



**AGRICULTURAL RESEARCH INSTITUTE  
PUSA**





# THE JOURNAL OF HYGIENE

EDITED BY

GEORGE H. F. NUTTALL, M.D., PH.D., Sc.D., F.R.S.

QUICK PROFESSOR OF BIOLOGY IN THE UNIVERSITY OF CAMBRIDGE

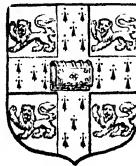
IN CONJUNCTION WITH

JOHN S. HALDANE, M.D., LL.D., F.R.S. ARTHUR NEWSHOLME, M.D., F.R.C.P.  
READER IN PHYSIOLOGY IN THE MEDICAL OFFICER TO THE  
UNIVERSITY OF OXFORD LOCAL GOVERNMENT BOARD

CHARLES J. MARTIN, M.B., D.Sc., F.R.S.

DIRECTOR OF THE LISTER INSTITUTE OF PREVENTIVE MEDICINE, LONDON

Volume IX. 1909



CAMBRIDGE  
AT THE UNIVERSITY PRESS

LONDON: FETTER LANE, C. F. CLAY, MANAGER  
AND H. K. LEWIS, GOWER STREET

EDINBURGH: 100, PRINCES STREET

BERLIN: A. ASHER & CO.

LEIPSIC: BROCKHAUS \*

NEW YORK: G. P. PUTNAM'S SONS

BOMBAY AND CALCUTTA: MACMILLAN & CO., LTD.

Entered at New York Post Office as Second Class Matter

[All rights reserved]

**Cambridge:**  
PRINTED BY JOHN CLAY, M.A.  
AT THE UNIVERSITY PRESS

## CONTENTS.

### No. 1 (April).

	PAGE
BLUE, R. Anti-plague Measures in San Francisco, California, U.S.A. . . . .	1
SYMMERS, W. ST C. AND WILSON, W. J. Some points bearing on the Bacteriology of Cerebro-spinal Meningitis . . . . .	9
DURHAM, H. E. Studies on Cider and Perry. No. I. Sulphite Preservatives . . . . .	17
WILSON, A. On the Relative Efficacy of the Doulton, Berkefeld and Brownlow Filters . . . . .	33
BULLOCH, W. AND CRAW, J. A. On the Transmission of Air and Micro-organisms through Berkefeld Filters. (Plate I. and One Text Figure) . . . . .	35
CRAW, J. A. On the Danysz Effect with reference to the Toxin- antitoxin Reaction. (One Figure) . . . . .	46
GIMLETTE, J. D. Plague in Further India . . . . .	60
TODD, C. An Anti-serum for Scorpion Venom . . . . .	69
MACCONKEY, A. Further Observations on the Differentiation of Lactose-fermenting Bacilli, with special reference to those of Intestinal Origin . . . . .	86
ARKWRIGHT, J. A. Varieties of the Meningococcus with special reference to a Comparison of Strains from Epidemic and Sporadic Sources . . . . .	104
GOADBY, K. A Note on Experimental Lead Poisoning . . . . .	122
PUBLICATIONS RECEIVED . . . . .	134

### No. 2 (September).

NASH, J. T. C. House Flies as Carriers of Disease . . . . .	141
HALLIBURTON, W. D. The Bleaching of Flour . . . . .	170
NOON, L. Observations on the Evolution of Immunity in Disease. (Fifteen Figures) . . . . .	181
KAUFMANN, W. P. On the Absence of Respiratory Disorders in those inhaling Starch-Dust over Long Periods . . . . .	220

*Contents*

	PAGE
LANE-CLAYPON, JANET E. Observations on the Influence of Heating upon the Nutrient Value of Milk as an Exclusive Diet for Young Animals. (One Figure) . . . . .	233
LANE-CLAYPON, JANET E. Multiplication of Bacteria and the Influence of Temperature and some other Conditions thereon. (One Figure) . . . . .	239
ARMIT, H. W. The Physiological Effect of Cobalt Carbonyl Vapour . . . . .	249
PUBLICATIONS RECEIVED . . . . .	252

*No. 3 (November).*

SCHRYVER, S. B. Some Investigations on the Toxicology of Tin, with Special Reference to the Metallic Contamination of Canned Foods . . . . .	253
BOYCOTT, A. E. AND HALDANE, J. S. The Progress of Ankylostomiasis in Cornwall . . . . .	264
HEWLETT, R. TANNER, VILLAR, SIDNEY AND REVIS, CECIL. On the Nature of the Cellular Elements present in Milk . . . . .	271
PAGE, CECIL H. W. British Industrial Anthrax. Part I. (One Figure) . . . . .	279
WILSON, W. JAMES. On Heterologous Agglutinins more particularly those present in the Blood Serum of Cerebro-Spinal Fever and Typhus Fever Cases . . . . .	316
MASSEY, R. E. A Comparison between the Germicidal Power of a Disinfectant in Solution and in the Emulsified State. (One Text Figure) . . . . .	341
Miscellanea . . . . .	347

*No. 4 (December).*

PAGE, CECIL H. W. British Industrial Anthrax. Part II. . . . .	357
SÜDMERSEN, H. J. AND GLENNY, A. T. Variation in Susceptibility of Guinea-pigs to Diphtheria Toxin. (Seven Charts) . . . . .	399
ARKWRIGHT, J. A. On the Production of Antitoxin by the Injection of Filtrates of Cultures of Non-virulent Diphtheria Bacilli . . . . .	409
JOHNSTONE, JAMES. Routine Methods of Shellfish Examination with Reference to Sewage Pollution . . . . .	412
PUBLICATIONS RECEIVED . . . . .	441
INDEX OF AUTHORS . . . . .	449
INDEX OF SUBJECTS . . . . .	451

## ERRATA

Vol. IX, No. 2

- p. 221, line 11 from below *read* "Beaumé" for "Beor."  
last line *delete* "-white."
- p. 222, line 6 *read* "boxing houses" for "boxing horses."
- p. 223, lines 18 and 21 *read* "kilogrammes" for "grains."
- p. 230, line 3 from below *read* " $[\alpha]_D=170^\circ$ " for " $[\alpha]$ ."



## ANTI-PLAQUE MEASURES IN SAN FRANCISCO, CALIFORNIA, U.S.A.

By RUPERT BLUE,

*Passed Assistant Surgeon, U.S. Public Health and Marine Hospital Service ;  
Commanding Plague Suppressive Campaign.*

THE plague suppressive campaign which is now drawing to a close in San Francisco has been conducted almost entirely on the principle that the great factor in the spread and continuance of the disease is the rat. The fact that the epidemic among human beings ceased within four months after the beginning of active operations and that in spite of the distribution of the disease in almost every quarter of the city, only one hundred and fifty-nine cases, with seventy-seven deaths occurred, proves the wisdom of this policy. The campaign has been conducted by the United States Public Health and Marine Hospital Service.

The previous epidemic of plague existed from March, 1900, to February, 1904, although it is probable that cases occurred prior to the first date. It is thought that the disease was imported from Hong Kong. It was suspected among the Chinese as early as 1896 or 1898, but this was not proven at that time. In all probability it existed among the Chinese prior to 1900, but they were able to hide the few cases owing to the fact that they were treated by physicians of their own nationality. In 1900, when a white Inspector of the Dead was put on duty in Chinatown, the discovery of the disease was made.

The source of infection of the 1907 outbreak was in all probability a recrudescence from an old focus. During the previous epidemic cases were found outside the city of San Francisco, in Oakland and in Contra Costa County, across the Bay, and in 1906 a case was found in Oakland.

## *Plague in San Francisco*

In all probability the infection in this instance was received from a ground squirrel (*Citellus beecheyi*), as there has undoubtedly been a ground squirrel enzootic of plague in Contra Costa County for several years. Four human cases occurred there in the previous epidemic and two during the summer of 1908. Infected ground squirrels and rats have been found in that section of country recently.

This was the second epidemic of bubonic plague in San Francisco, the previous outbreak having been confined to the Chinese quarter. The present epidemic began May 27, 1907, a little over a year after the great fire and earthquake, but no cases were discovered between that time and mid-August when the disease began to appear in various parts of the city. It should be noted that San Francisco has always had a great many fleas, but that during the summer and autumn of 1907 they were unusually prevalent, also that on account of the great catastrophe sanitary conditions were unusually bad in the city at that time.

The following tables show the incidence of human plague and the races attacked :

### *Epidemic Plague.*

				Cases	Deaths
	1907				
May	...	...	...	1	1
August	...	...	...	18	6
September	...	...	...	55	25
October	...	...	...	34	25
November	...	...	...	41	12
December	...	...	...	18	7
	1908				
January	...	...	...	2	1
			Total	159	77

### *Races attacked.*

Americans	...	...	...	...	79
Europeans	...	...	...	...	66
Chinese	...	...	...	...	8
Japanese	...	...	...	...	5
African	...	...	...	...	1
					159

The last case of human plague occurred January 30, 1908.

The following table shows the number of human cases per month and the meteorological conditions during that time:

Month	Cases	Deaths	Average temperature	Rainfall in inches	Character of day
1907 May	1	1	56·3° F.	.04	{ Clear 10 Part cloudy 17 Cloudy 4
June	0	0	57·2° F.	1·28	{ Clear 10 Part cloudy 14 Cloudy 6
July	0	0	58·0° F.	Trace	{ Clear 5 Part cloudy 17 Cloudy 9
August	18	6	59·3° F.	.02	{ Clear 11 Part cloudy 16 Cloudy 4
September	55	25	60·6° F.	.11	{ Clear 13 Part cloudy 15 Cloudy 2
October	34	25	60·6° F.	1·36	{ Clear 10 Part cloudy 10 Cloudy 11
November	41	12	57·8° F.	.04	{ Clear 14 Part cloudy 13 Cloudy 3
December	13	7	52·4° F.	3·66	{ Clear 6 Part cloudy 11 Cloudy 14
1908 January	2	1	50·8° F.	4·88	{ Clear 5 Part cloudy 11 Cloudy 15

It will be noted that the greatest number of human cases occurred during the warm, dry months (August 1st to December 1st) i.e. during the period of greatest flea prevalence. Inasmuch as no effective work was done until September 25th and no great uniformity of action was secured until mid-November these figures may be taken as representing natural conditions.

The rats examined for September, 1907, were very largely collected from the infected districts, the remaining months give a truer picture of the condition of the entire rat population. It will be noted that while the incidence of human plague was greatest in the warm, dry months, rat plague was greatest in the cold, wet months. It is believed that this is explained by the fact that the rats congregate in their burrows to avoid the cold and wet and that their close proximity permits one infected rodent to distribute *Bacillus pestis*-laden fleas to all the other inhabitants of the warren.

## Plague in San Francisco

The following table shows the incidence of rodent plague and its relation to meteorological conditions:

Month	Number examined	Number infected	Per cent.	Average Temperature	Rainfall in inches	Character of day
September	1002	27	2·69	60·6° F.	.11	{ Clear 13 Part cloudy 15 Cloudy 2
October	2679	23	.86	60·6° F.	1·36	{ Clear 10 Part cloudy 10 Cloudy 11
November	3954	36	.88	57·8° F.	.04	{ Clear 14 Part cloudy 13 Cloudy 3
December	4308	48	1·11	52·4° F.	3·66	{ Clear 6 Part cloudy 11 Cloudy 14
January	6622	70	1·05	50·8° F.	4·88	{ Clear 5 Part cloudy 11 Cloudy 15
February	11700	45	.38	51·0° F.	5·39	{ Clear 11 Part cloudy 12 Cloudy 6
March	19263	52	.26	54·8° F.	.90	{ Clear 20 Part cloudy 10 Cloudy 1
April	15524	34	.21	56·3° F.	.22	{ Clear 17 Part cloudy 10 Cloudy 3
May	11311	20	.18	55·4° F.	.76	{ Clear 17 Part cloudy 12 Cloudy 2
June	18624	4	.02	55·3° F.	.01	{ Clear 16 Part cloudy 9 Cloudy 5
July	11204	2	.017	57·4° F.	.02	{ Clear 11 Part cloudy 17 Cloudy 3
August	10988	0	0	57·3° F.	.01	{ Clear 11 Part cloudy 10 Cloudy 10
September	15902	0	0	59·3° F.	.29	{ Clear 16 Part cloudy 9 Cloudy 5
October	10178	2	.019	58·8° F.	.661	{ Clear 16 Part cloudy 7 Cloudy 8

All rodents captured or found dead were identified as to sex and species and were carefully tagged to show where, when and by whom captured. The following table shows the result of these identifications:

Total No. rats identified <sup>1</sup>	...	...	189016
<i>Mus norvegicus (=decumanus)</i>	...	...	152760
<i>Mus rutilus</i> ...	...	...	3291
<i>Mus musculus</i>	...	...	32941
<i>Mus alexandrinus</i>	...	...	24
No. examined bacteriologically	...	...	138259
No. infected with <i>B. pestis</i>	...	...	368

Rats taken alive were chloroformed and combed for fleas. The fleas from each individual rat were placed in a separate vial containing 70% alcohol. These were delivered to the laboratory where they were identified by an entomologist. Of 10,972 rat fleas examined the following species were identified:

<i>Ceratophyllus fasciatus</i> <sup>2</sup>	...	...	68.07 %
<i>Pulex cheopis</i>	...	...	21.36
<i>Pulex irritans</i>	...	...	5.57
<i>Ctenopsyllus musculi</i>	...	...	4.48
<i>Ctenocephalus canis</i>	...	...	.52

The *Ceratophyllus fasciatus* is the common rat-flea at all seasons on this coast. In one of the twelve sanitary districts of the city, however, the *Pulex cheopis* was most abundant from January to June as follows:

Of 1,153 determinations there were,

<i>Pulex cheopis</i>	...	...	...	67.82 %
<i>Ceratophyllus fasciatus</i>	...	...	...	30.78
<i>Pulex irritans</i>	...	...	...	.78
<i>Ctenocephalus canis</i>	...	...	...	.26
<i>Ctenopsyllus musculi</i>	...	...	...	.35

Plague foci were recorded in this district as follows:

Epidemic	...	...	...	...	4
Epizootic	...	...	...	...	38
No. of blocks infected as shown by human and rodent cases	...	...	...	...	21
Total rat catch from November 9th to June 30th					15,114

<sup>1</sup> To November 1, 1908.

<sup>2</sup> Specimens of these fleas were kindly sent to Cambridge by Dr Rupert Blue and were submitted to the Hon. N. Charles Rothschild who confirmed the determinations.—G.H.F.N.

## Plague in San Francisco

Rats trapped in insanitary basements and stables had the greatest number of parasites. Sickly and very young rats showed a high degree of infestation. Sewer rats, as a rule, had few fleas. In the winter, from January to May (the off-season of plague) there were few, if any, fleas found upon the rats examined. In a count of twenty selected at random, not a single specimen was obtained. In Oakland, as late as June 15th, the same conditions were noted. On the 15th one hundred and eighty-seven rats (*Mus decumanus*) were chloroformed and combed, one hundred and forty-five rats were infested with five hundred and ninety-nine fleas and forty-two rats had no fleas. Ovulation was first noted in May.

### Fleas from Human Hosts.

	Male	Female	Total
<i>Pulex irritans</i>	500	764	1264
<i>Ceratophyllus fasciatus</i>	—	4	4
<i>Ctenocephalus canis</i>	2	1	3
1271			

Of the 1271 fleas taken from the human host in San Francisco not one was a *cheopis* and only four were *Ceratophyllus fasciatus*. The hosts were the labourers engaged in the plague suppressive measures.

Although there were employed at one time over one thousand men, most of whom were in very close contact with rats, constantly entering buildings from which cases of human plague had been taken, no case of plague occurred among them. One morgue attendant, however, was stricken with the disease, probably by receiving infected fleas from the corpse he was removing. A physician and a nurse contracted the disease while in the discharge of their duty in the City and County Hospital. Bubo-septicaemic plague was admitted to the general wards of the hospital by mistake, no precautions being taken before the seizure of the doctor and nurse occurred. Both recovered. Precautions taken by the men consisted in wearing heavy clothing with high shoes, the trousers being tied to the leg by means of pieces of string. Almost all of the men wore oiled-leather gloves with gauntlets. When handling rats which had not been immersed in corrosive sublimate rubber gloves were worn or the rats handled with tongs.

In the laboratory great care was taken to prevent the rat-skinners from becoming infected. All rats were immersed in a corrosive sublimate solution before tacking them on the shingles, and rubber gloves worn by the rat-skinners. Rats were forwarded in heavy

galvanised iron cans having tight fitting covers, and were stretched on shingles. The tag was read and recorded in a book and the rat passed to specially trained skinners who grew very expert in recognizing the macroscopic lesions of plague. They laid aside those which they considered most suspicious. All rats were carefully gone over by a bacteriologist and pathologist, special attention being paid to those set aside by the laboratory attendants. In the event of finding lesions which resembled plague, smears, cultures and inoculations into guinea-pigs were made.

In addition to plague many interesting pathological lesions were found. Among the animal parasites observed the *Trichinella spiralis* and the *Hymenolepsis diminuta* are also parasitic for man. The *Trypanosoma lewisi*, *Trichosomum crassicaudum*, *Hymenolepis nana* were also observed. Scabies was noted as a very common condition. Among the organic lesions encountered may be mentioned dilatation of the pericardium, caseating abscesses of the lungs, hob-nail liver, nephritis, vesical calculi and tumor growths such as lipomata, fibromata, adenomata, sarcomata and carcinomata. Eighty cases of the leprosy-like disease of rats were found. There seems to be some connection between this disease and places where cattle are slaughtered or meat sold, but it has been impossible to decide how great a factor the meat industry may be in this disease.

The means by which the *Bacillus pestis* maintains its existence in quiescent periods has had considerable attention during the present epidemic and the conclusion has been reached that it is through a chain of acute enzootic foci in sewers and other inaccessible places. Inasmuch as no case of chronic plague, as described by the British Commission in India, was found out of 138,259 rats examined, it is not thought that chronic plague plays any great part in the continuance of the disease in San Francisco. This may possibly be explained by the fact that there seems to be some difference in virulence between the strain of *Bacillus pestis* found here and that recovered in Bombay. It has been observed that the present San Francisco strain does not lose its virulence on artificial media, but it has been the observation of many who have worked with the Bombay strain that in a few months it becomes weakened. This may account for the occurrence of chronic plague in India and not in San Francisco. As further evidence of the continuance of the disease by acute plague may be cited the fact that on tearing up wooden flooring and similar harbouring places extensive rat catacombs have been found, in many cases containing large numbers of rodent

cadavers. These vary from fresh bodies to mummified carcasses, showing that the epizootic has ranged over a considerable time. In almost all such cases in which bacteriological examination was possible they were found to have died of plague. This would seem proof that plague epizootics were continued in inaccessible and undiscovered places.

Experiments were made to determine how long rats would live under unfavourable conditions such as would be found on the freight trains, ships and other common carriers leaving the Pacific Coast. Without water or food of any sort, the maximum duration of life was five days, three rats being used in the experiment. On a diet of dried grain (wheat) entirely without water, the experiment being made on three young rats, the maximum time before death was fifteen days, the minimum, eight days. On a diet of bread and meat, without water, three rats on which the experiment was made were alive on the thirtieth day.

The plague eradication measures may be briefly summarised as follows:

A simultaneous attack upon the habitation and food supply of the rat.

The destruction of rat burrows and nesting places.

The separation of the rat from his food supply by concreting and screening such places as stables, warehouses, markets, restaurants, etc.

The prevention of the entry of the rat into human habitations by the use of concrete or other impervious material on the ground area or by elevating the building so as to allow free access to the natural enemies of the rat beneath the same.

Disinfection of rat burrows by the use of strong antiseptic solutions and chloride of lime in places likely to furnish fleas.

Disinfection of buildings in which either human or rodent cases have occurred. This latter measure is not considered as important as rat extermination. All the human cases were isolated in a rat-proof compound.

## SOME POINTS BEARING ON THE BACTERIOLOGY OF CEREBRO-SPINAL MENINGITIS.

BY W. ST CLAIR SYMMERS, M.B. (ABERD.)

*Musgrave Professor of Pathology, Queen's College, Belfast*

AND W. JAMES WILSON, M.D. (R. U. I.)

*Joint-Lecturer on Sanitary Science, Queen's College, Belfast.*

*Fermentative activity of the Diplococcus intracellularis meningitidis (Weichselbaum).*

IN determining the fermentative powers of this organism both fluid and solid cultures were used. *The fluid medium* was made up according to the formula employed by Dr M. H. Gordon. It consisted of Lemco ·1 gram, peptone ·1 gram, sodium bicarbonate ·1 gram, 10 per cent. watery solution of litmus 10 c.c., water to 100 c.c. This medium was sterilized by heating under pressure and to it was then added the substance to be investigated in the proportion of 1 per cent. Subsequent sterilisation was effected by heating in the steamer for 10 minutes on three successive days. In the case of laevulose, arabinose, and xylose the flasks were heated over the Bunsen flame, as in the steamer the medium turned red.

To each 500 c.c. of the medium 50 c.c. of sterile ascitic fluid were added. The medium was then put into tubes which were incubated at 37° C. for three days to see that they were sterile. It was found that in this fluid the meningococcus grew luxuriantly, a pellicle forming on the surface of the bouillon.

Most of the strains of meningococci used were isolated from cases occurring in Belfast and were in cultivation for various lengths of time, some being isolated only a few days, others having been in the laboratory for over a year. In addition to the Belfast strains we had three cultures obtained from the Hygienic Institute, Hamburg. The cultures were examined daily and the colour of the medium was carefully compared with controls, the tubes being examined against a white background.

In no medium was any gas produced. Glucose, maltose, and dextrin were fermented with the production of acid, whilst no fermentation of any other substance was observed. With glucose and maltose in nearly all cases the medium became red and remained red, in a few cases bleaching preceded the reddening. With dextrin the medium became red, it was then bleached and finally, about the end of a fortnight, became blue again.

With the various other substances the medium remained blue; sometimes bleaching occurred but the blue colour returned when the tubes were left all night at room temperature.

After each of the substances in the following list is placed the number of strains of meningococci employed in determining the fermentability of the substance :—Glucose 60, Laevulose 43, Galactose 53, Maltose 59, Saccharose 47, Dextrin 31, Lactose 20, Inulin 23, Arabinose 20, Raffinose 19, Glycerine 25, Erythrone 17, Mannite 29, Dulcite 8, Rhamnose 15, Sorbite 14, Xylose 5, Adonite 21, Salicin 32, and Amygdalin 15.

The media were all prepared in the same manner and the same ascitic fluid was used in all cases, so that the conditions were uniform throughout the series.

We desire to call especial attention to the negative results we obtained with laevulose and galactose. We used galactose prepared by Merck and by Kahlbaum.

The results of these fermentation tests confirm those obtained by von Lingelsheim and by ourselves in previous experiments. Sheenan and Ritchie at Edinburgh have obtained similar results. On the other hand, Gordon, Buchanan, Rundle, Mottram and Williams, and Arkwright have obtained positive results as regards the fermentation of galactose and laevulose.

In solid media also we got no fermentation of laevulose and galactose.

*Solid media.* Nutrient agar was made containing 3 per cent. of Chapoteaut's peptone and to it was added 1 per cent. of the sugar and some litmus solution. Sterilisation was effected by steaming for 10 minutes on 3 successive days. To 2 parts of this medium, which had been melted and cooled to 50° C., 1 part of sterile ascitic fluid was added. The medium was then put into tubes which were placed in a slanting position.

After the agar had set firmly the tubes were incubated for 2 days at 37° C. and those that were contaminated were discarded.

The action of ten strains of meningococci was tested on media which had been thus prepared and which contained respectively glucose, laevulose, galactose and dextrin.

In the case of glucose permanent reddening of the medium in the neighbourhood of the condensation fluid occurred—with dextrin at the same place the medium first became red and then became blue again. In the case of laevulose and galactose the medium and condensation fluid remained blue.

*Fermentative activity of Gram-negative cocci isolated from cases of sporadic cerebro-spinal meningitis.*

The same media were used as in the previous experiments.

The results obtained with 10 Gram-negative cocci isolated from cases of sporadic cerebro-spinal meningitis (and which were proved by Houston and Rankin to differ from Weichselbaum's cocci in respect of their agglutinins and opsonins) are shown in the following table:—

Number of strains	Glucose	Laevulose	Galactose	Maltose	Saccharose
8 strains	+	-	-	+	-
1 strain	-	-	-	+	-
1 strain	-	-	-	-	-

+ indicates production of acid.

- indicates no change in reaction.

It is evident from this Table that 8 of these cocci are identical with Weichselbaum's coccus as regards fermentative power; of the remaining two, one differs from the true meningococcus in not fermenting glucose though able to ferment maltose, the other had no fermentative activity, though in morphology, feeble vitality, and appearance of its growth it closely resembled the meningococcus. In its absence of fermentative powers it resembles the *Micrococcus catarrhalis*.

From the above investigation it would appear that Weichselbaum's diplococcus has constant fermentative characters whilst there is some variability among the Gram-negative cocci occurring in sporadic cases of cerebro-spinal meningitis.

*Characters of certain other Gram-negative cocci occurring in cerebro-spinal fluid.*

In addition to Weichselbaum's and Still's cocci there are sometimes met with in cerebro-spinal fluid Gram-negative cocci which belong to

## Cerebro-spinal Meningitis

an entirely different class. The members of this class grow well on ordinary media both at 20° C. and at 37° C., survive for several weeks without transplantation and finally on the Drigalski-Conradi medium give an abundant growth. In morphology also they differ from the meningococci. They show no tendency to tetrad formation but rather to formation of short chains consisting of four or six individuals, though the diplococcal is the commonest arrangement. Moreover the organisms stain well, there being no evidence of autolysis. In cultures on both solid and fluid media among the diplococci short bacillary forms and even unsegmented uniformly staining threads 20 $\mu$  in length are frequently seen. It is quite certain that these bacillary forms and threads are not contaminations but represent variant forms of the organism.

We may here note that D'Este Emery found extremely pleomorphic diplococci in the cerebro-spinal fluid of three cases of posterior basic meningitis which, though in some respects differing from the ones here described, agreed with them in the assumption at times of the bacillary form.

We have met with this class of organism on four occasions. In one case it appeared to be the only organism present in the cerebro-spinal fluid, in two cases it was associated with Weichselbaum's diplococcus, in the fourth, a case of posterior basic meningitis, it was associated with a Gram-negative coccus which, though in most respects resembling the meningococcus, produced no acid in media containing glucose and maltose.

These micro-organisms had no action on glucose, laevulose, galactose, maltose, lactose and saccharose.

Their growth on ascitic agar was somewhat more opaque than that of the meningococcus.

On the Drigalski-Conradi medium we find that Weichselbaum's and Still's meningococci and Pfeiffer's *Micrococcus catarrhalis* (culture obtained from Král) exhibit no growth.

### *Cerebro-spinal meningitis due to Gram-positive cocci.*

On seven occasions we have obtained cultures of Gram-positive diplococci from the cerebro-spinal fluid. One of these organisms had the characteristic lanceolate shape of the pneumococcus and possessed a capsule; all the others were found to be non-capsulated when the sediment from the cerebro-spinal fluid was examined. From the fermentative activity of the other six when grown in Gordon's nine media it appeared

that four of them were probably members of the *Streptococcus faecalis* group. Another one was probably a member of the *Streptococcus salivarius* group. The 7th had a thick moist growth on agar quite different from that of streptococci and somewhat like that of the meningococcus but more opaque. Its vitality was good as it survived several weeks without subculture. Morphologically it showed large Gram-positive diplococci resembling in size and shape the giant forms occurring in cultures of the meningococcus.

A full account of these organisms has been given in a previous communication (1907) in which we also point out that the post-mortem findings in these cases may be identical with those met with in cases of cerebro-spinal fever.

On one occasion we found the *Bacillus anthracis* responsible for a haemorrhagic lepto-meningitis.

From the fibrino-purulent cerebro-spinal fluid of another case we isolated the *Bacillus typhosus* in pure culture.

From a third case we obtained a culture of the *Bacillus enteritidis* (Gaertner). The proof that in these latter two cases we were dealing with the typhoid bacillus and with Gaertner's bacillus was established by a consideration of the cultural, fermentative and morphological characters of the bacilli, as well as by agglutination and saturation experiments.

#### *Agglutination of meningococci.*

On 47 occasions the agglutinative effect of the blood serum of cerebro-spinal fever patients was investigated. The blood was taken from patients in all stages of the disease as well as from those who were convalescent.

An emulsion in normal salt solution was made from a 12—24 hours' culture of the meningococcus. With this emulsion 1 in 10, 1 in 20, 1 in 50 and 1 in 100 dilutions of the blood serum were made in Wright's capillary pipettes. Finally the pipettes were sealed and incubated for 2 hours at 37° C. At the end of this time they were examined for clumping both by the naked eye and the microscope.

According to the degree of the agglutination we have applied the terms small, medium sized and large to the clumps.

On three occasions clumping visible to the naked eye occurred instantaneously on making the 1 in 10 dilution.

Control tests were carried out with serum taken from normal individuals or from typhoid fever patients; the results were that with

*Cerebro-spinal Meningitis*

a 1 in 5 dilution of the serum medium sized clumps formed, with a 1 in 10 dilution either no clumps or very small ones.

With a 1 in 10 dilution	45 sera	gave large clumps.
" "	1 serum	medium sized clumps.
" "	1 "	small "
With a 1 in 20 dilution	19 sera	large clumps.
" "	6 "	medium clumps.
" "	10 "	small "
" "	12 "	no clumps.
With a 1 in 50 dilution	4 "	large clumps.
" "	7 "	medium clumps.
" "	5 "	small "
" "	31 "	no clumps.
With a 1 in 100 dilution	0 "	large clumps.
" "	2 "	medium clumps.
" "	4 "	small "
" "	41 "	no clumps.

*Different degree of agglutinability of old and young cultures of the meningococcus.*

We have found that meningococci that have been in cultivation for a long time are much more readily agglutinated than recently isolated cocci not only by immune serum but also by normal serum. Roughly stated, old avirulent cultures of the meningococcus are twice as easily agglutinated as virulent cultures, when the dilutions herein mentioned are used.

For example a sample of Flexner and Jobling's serum which agglutinated a recently isolated culture of the meningococcus in a dilution of 1 in 300, caused an equal degree of agglutination with an old avirulent culture in a dilution of 1 in 600. Similarly the serum of a patient which agglutinated an old culture in a 1 in 50 dilution caused a similar amount of agglutination in the case of a recently isolated culture in a 1 in 20 dilution only.

*Difference between old and young cultures of the meningococcus with reference to the opsonic index.*

Houston and Rankin have shown that old cultures of the meningococcus are readily phagocytosed when acted on by normal serum, whereas with young cultures in the same conditions phagocytosis is very feeble.

An experiment which we made seemed to indicate that old cultures are able to absorb from a serum both normal and immune opsonins whilst young cultures combine only with immune opsonin.

Serum taken from a cerebro-spinal fever patient was divided into two equal portions, one portion was saturated with several loopfuls of growth taken from a young culture of the meningococcus on Chapasgar<sup>1</sup>, the other was saturated with an equal quantity of growth from an old culture. The sera were left for two hours at 30° C. and then for two hours at room temperature. They were then centrifugalised and the opsonising effect of the clear supernatant serum was determined on an old and young culture of the meningococcus. The serum that had been saturated by the old culture became completely devoid of opsonising action both on old and young cultures; the serum on the other hand that had been saturated with the young culture still caused marked phagocytosis of an old culture. The above deduction, drawn from a single experiment, we regard as merely indicative of what is to be expected on further investigation.

*Agglutinating action of the blood serum of cases of cerebro-spinal fever on bacilli of the typhoid, colon and alkaligenes groups.*

In a previous communication (1908) we have pointed out that the blood serum of cerebro-spinal fever patients occasionally agglutinates the typhoid and colon bacillus and that it almost constantly agglutinates an organism closely related to *Bacillus faecalis alkaligenes* which was isolated by us from Belfast tap water and to which we gave the name *Bacillus Grosvenor*. Later observations have confirmed the frequency of this phenomenon, but we have found that it is not invariably present, some cases having failed to show the reaction though examined on several occasions.

Since the paper above referred to was published we have used as additional controls the blood serum of nine patients suffering from typhus fever, three cases of meningitis (not meningococcal), and one of pneumonia, and in no case was there agglutination in a dilution of 1 in 50. With no blood except that taken from cases of cerebro-spinal fever have we got agglutination of the Grosvenor bacillus in higher dilutions than 1 in 100 although 168 specimens have been examined.

<sup>1</sup> This medium consists of:

3 % agar (made with Chapoteaut's peptone)	...	...	2 parts.
Raw ascitic fluid	...	...	1 part.

In 16 cases of cerebro-spinal fever the blood serum gave marked clumping within an hour to dilutions of 1 in 1000, and further we found that one of the 16 agglutinated in 1 in 1400, two in 1 in 1500, four in 1 in 1600, and four in 1 in 2000 dilutions respectively.

We have shown that it is possible to remove the agglutinins from the serum by saturation with the Grosvenor bacillus, whilst saturation with the *Meningococcus*, *Bacillus typhosus*, *B. coli communis* or *B. faecalis alkaligenes* (Král) fails to do so.

The agglutinins for the Grosvenor bacillus and for the meningococcus are quite distinct. Heating the serum for 10 minutes at 65° C. completely destroys the agglutinins for the Grosvenor bacillus.

By means of precipitation with various strengths of ammonium sulphate solution it was found that these agglutinins were associated with the globulin component of the serum and moreover that it was that portion known as the "pseudo-globulin" fraction which contained them in greatest amount.

As we have never succeeded in cultivating *Bacillus Grosvenor* from the bodies of any of the patients we can advance no explanations of the phenomenon and content ourselves with merely stating the facts.

#### REFERENCES.

- ARKWRIGHT, J. A. (1907). *Journ. of Hygiene*, vii. p. 193.
- BUCHANAN, R. M. (8. vi. 1907). *Lancet*, i. p. 1590.
- EMERY, W. D'ESTE (27. VIII. 1904). *Lancet*, ii. p. 593.
- GORDON, M. H. *Report to the Local Govt. Board on the Micrococcus of epidemic cerebro-spinal meningitis etc.* Feb. 1907.
- HOUSTON, T. and RANKIN, J. C. (16. xi. 1907). *Brit. Med. Journ.* ii. p. 1414.
- VON LINGELSHHEIM (1906). *Klin. Jahrbuch*, xv. Heft 2.
- RUNDLE, MOTTRAM, and WILLIAMS (27. VII. 1907). *Lancet*, ii. p. 220.
- SHEENAN, T. and RITCHIE, W. T. (1908). *Journ. of Pathol. and Bacteriol.* XII. p. 456.
- SYMMERS, W. St C. and WILSON, W. J. (22. VI. 1907). *Brit. Med. Journ.* i. p. 1477.
- SYMMERS, W. St C. and WILSON, W. J. (1908). *Journ. of Hygiene*, viii. p. 313.
- WILSON, W. J. (28. XII. 1907). *Lancet*, ii. p. 1816.
- WILSON, W. J. (13. VI. 1908). *Lancet*, i. p. 1686.
- WILSON, W. J. (20. VI. 1908). *Lancet*, i. p. 1796.

## STUDIES ON CIDER AND PERRY.

### No. I. SULPHITE PRESERVATIVES.

By HERBERT E. DURHAM, M.A., M.B., B.C. (CANTAB.),  
F.R.C.S. (ENG.), *Hereford.*

THE practice of using preservatives in the process of making cider is very widely spread, and moreover is much encouraged by the makers of the preservatives.

There are three chief reasons, or perhaps one should rather say excuses, for the use of preservatives; the first is to prevent the complete fermentation of the sugar in the must—in popular language “to preserve the sweets,”—the second to cure or prevent “diseases” of fermentation, and the third (when sulphites are used) to prevent the blackening which occurs with certain kinds of apple. The nature and cause of this blackening is not fully understood, and I hope in a further communication to throw some light on it.

It would appear that some years ago salicylic acid was the substance favoured by cider makers in this country, as benzoic acid (or its salts) is now in favour in the United States. From my analyses of representative samples of the products of the industry in this country, it seems that sulphite preparations are now more in vogue. In part, this change may be due to the attention which has been drawn to the presence of added salicylic acid in ciders, but perhaps it is still more due to the excuse that sulphurous acid is not “objectionable.” Thus whilst some makers only go so far as to say that their products contain no objectionable preservative, at least one maker has not scrupled to state that his cider contains no added chemicals, although it contains a considerable percentage of sulphurous acid.

According to Laborde<sup>1</sup> and Warcollier<sup>2</sup>, complete sterilisation of the must is only secured by the addition of 500 milligrams of sulphur

<sup>1</sup> Laborde, *Cours d’Oenologie*, Paris, 1908, p. 265.

<sup>2</sup> Warcollier, *Le Cidre et le Poiré*, 20<sup>e</sup> Année, Paris, 1908, p. 202.

dioxide per litre; but smaller doses have more or less considerable inhibitory effect.

Pending the promised report of the Department of Agriculture, Washington, U.S.A., there does not appear to be much direct experiment on the deleterious action of sulphurous acid on man. Leuch (*vide Laborde*) has shown from actual trials that a single dose of wine (300 c.c., about  $\frac{1}{2}$  pint) containing 50 mgrms. of free, or 300 mgrms. of combined, sulphurous acid per litre gave rise to discomfort; but the effect of repeated doses does not appear to have been tried. These observations led to the assumption that "free" sulphurous acid was five times more deleterious than the "combined," and have led to legislation on the subject. It is interesting to observe that, according to Laborde, the first regulation of this antiseptic in France was undertaken on account of heavily sulphited English beer which was imported into Paris. Besides the deleterious action of the acid in undue quantity, the use of this and other preservatives undoubtedly assists the manufacture of fraudulent concoctions which masquerade under the title of cider or wine. It may be concluded that the most recent French law on the limitation has been introduced to minimise such frauds, which have done so much harm to the French wine industry; and it may be remarked that this law has reduced the permissible amount of sulphur dioxide to less than a third of that previously allowed.

Another reason against the use of sulphur dioxide is the effect on flavour of the product. Warcollier (*loc. cit. supra*) says that 30—40 mgrms. per litre are recognisable, if recently added to cider; but, if added to the must before fermentation considerably larger doses are not detected. It is worth while mentioning that some of the samples, which I analysed, were submitted to a practised palate, and the prediction of the presence of the acid was confirmed chemically, except when only about 10 mgrms. per litre were present.

There are, then, three lines of argument against the unlimited use of sulphur dioxide in cider—detriment to health, assistance to fraudulent concoctions, and deterioration of flavour.

The retention of a portion of unfermented sugar in the finished product can be accomplished by the old process of repeatedly "racking"; "racking" consists in drawing off the liquid from the sediment of yeasts, and inasmuch as the yeasts are continually abstracting food materials (notably nitrogenous and phosphorised substances) from the must into themselves, the racking impoverishes the liquid of these all-important constituents, until there is not sufficient to support the further growth

of those yeasts which remained suspended in the fluid. The keeping quality of the cider then depends upon the lack of nutritive material combined with the preservative action of the carbonic acid gas and alcohol. It is worth noting here that in France the addition of "artificial" carbonic acid has to be declared.

In practice the effect of racking is often or even usually enhanced by using "matched" casks, that is to say the racked off liquid is received into casks in which a "match" of sulphur has been burnt. Thereby the mere mechanical action of the racking is aided to a more or less considerable degree by the antiseptic action of the sulphur dioxide. From this, the step to a deliberate addition of antiseptic (sulphurous acid, sulphites, salicylic acid, etc.) is a small one, especially as the labour involved is also lessened.

Another method of producing sugar-containing cider is the well known champagne process, in which the use of antiseptics is superfluous.

There is yet another method of obtaining a sweet, but not sugary, cider. This also does not involve the use of antiseptics for the sweetness is obtained by the use of artificial agents such as saccharin or dulcin. This process is still practised with apparent impunity in this country, but abroad, both in cider- and wine-making it is only carried on in defiance of the law. In this country there is a reprehensible laxity in regard to the use of these artificial sweetening agents, the use of which for these purposes is entirely fraudulent.

#### *Laws concerning use of sulphites, etc., in food products.*

(1) *United Kingdom.* In order to ascertain the state of the law in this country with regard to the use of sulphurous acid and sulphites, I enquired of my friend, Dr Buchanan, Inspector of Foods, Local Government Board. His reply dated 10/vii/1908 states that "in the United Kingdom there are no prescribed official limiting quantities of sulphurous acid or of sulphites permitted in foods or drinks."

Since there are no regulations, it is not surprising that an undue sense of security is given to the public by the recommendations or analyses of unofficial trading concerns. Thus I find that ciders have been given guarantees of "purity" although samples of the same make contain much sulphurous acid, in some cases more than the former French maximum of 200 milligrams per litre. Such guarantees of purity might perhaps be prevented if it were made obligatory to declare

the presence of preservatives not only on the package but also in the analytical report or certificate.

(2) *France.* The amount of sulphurous acid was first limited by law in France in 1901, the amount permitted in wines and cider being limited to 200 milligrams per litre, whether "free" or "combined." In 1907, the amount permitted was increased to 350 milligrams per litre, and it was also enacted that in cases where alkaline bisulphites were used, only 20 grams per hectolitre were allowed. This year, 1908, the permissible dose has again been altered, so that now only 100 milligrams of the acid (free or combined) per litre or 10 grams of the salts per hectolitre are allowed.

(3) *United States of America*<sup>1</sup>. There are special State Regulations and also Federal Rules; these latter apply only to interstate and export commerce.

The different States have various regulations; thus "several States, e.g. Wyoming, Indiana, North Carolina, North and South Dakota, Texas and Wisconsin prohibit the addition of sulphurous acid or compounds derived therefrom in foods and beverages." The Federal rules limited (see Food Inspection Decision 76, issued July 13, 1907) the amount of sulphur dioxide, subject to declaration, to 350 milligrams per litre provided that not more than 70 milligrams are in a free state. It was recognised that some regulation was needed, as is shown by the following extract (p. 9). "It is absolutely necessary to restrict in some manner the sulphur dioxide in cases in which it is used under conditions, such that it may enter into combination with acetaldehyde, sugars, etc., present in food products, and it is believed that under the restrictions suggested, the public will be protected from products unduly sulphured, during the period which must elapse before experimental evidence can be obtained, as to whether a total restriction in the use of sulphur dioxide, under all the conditions mentioned, is necessary on account of the toxic properties possessed by sulphur dioxide in the combined form."

It may be presumed that the limitation by this regulation was designed to permit the importation of French wines. According to rumour, difficulty arose through importation of more highly sulphited products than this decision allowed, and an amended decision is now in force (F.I.D. 89, issued March, 1908). So that pending determination by the Referee Board of consulting scientific experts, "no objection will

<sup>1</sup> I am indebted to Dr Buchanan of the Local Government Board and to Dr Wiley, Chief of the Bureau of Chemistry of the United States Department of Agriculture, for the information concerning the law in U.S.A.

be made to foods which contain the ordinary quantities of sulphur dioxid, if the fact that such foods have been so prepared is plainly stated on the label of each package." It may be noted here that benzoate of soda appears to be the favourite (or even "necessary") adulterant for cider; the limit allowed, with declaration, being 0·1 %. It is further provided (F.I.D. 76, p. 9) that foods containing such declared additions shall not be labelled as "guaranteed to conform with the Food and Drugs Acts."

(4) *Austria-Hungary* was the first country to prescribe limits, and in 1885 a limit of 20 milligrams was laid down; later it was increased to 80 mgrms. *Italy*, *Belgium*, and the *Argentine Republic* may be mentioned as other countries which have direct laws on the subject.

#### *Free and combined sulphurous acid.*

It has already been mentioned that sulphurous acid is recognised to take two forms in substances like wines, the "free" and the "combined."

The basis of this distinction appears to be that combinations of the acid which are not attacked by iodine are called "combined." To some extent this distinction is probably academical, since exposure to the free hydrochloric acid of the gastric juice would decompose the so-called "combined" acid.

The mode of estimating the "free" sulphurous acid in wines consists in a rapid direct titration of the acidified wine with standardised iodine solution and starch indicator, the operation being conducted under an atmosphere of carbonic acid gas<sup>1</sup>.

Dr Wiley informs me that direct titration with iodine is the official method in U.S.A.

#### *Direct titration with iodine.*

1. Dry cider free from SO<sub>2</sub> required addition of iodine equal to 0·166 % SO<sub>2</sub>.
2. Dry cider              ditto              ditto              0·014 % SO<sub>2</sub>.
3. Same sample after treatment with animal charcoal, ditto    0·008 % SO<sub>2</sub>.

It has been pointed out by Matthieu<sup>2</sup> that there are many substances in wines which are capable of taking up iodine besides any SO<sub>2</sub> which may be present, and that, so far as this adulteration is concerned, the

<sup>1</sup> Lunge, *Chemisch-technische Untersuchungsmethoden*, III. p. 622, Berlin, 1905.

<sup>2</sup> Quoted by Dujardin and Salleron, *Instruments de précision appliqués à l'oenologie*, 4th ed., p. 222. Paris.

method is quite fallacious. The same objection holds good for cider, as is well shown by some titrations which were made on samples in the manufacture of which no sulphuring whatever had been employed, and in which no appreciable amount of  $\text{SO}_2$  was revealed by the distillation method.

Animal charcoal causes a very complete removal of the tannins and other reducing matters in the cider, and the effect upon the amount of iodine required is marked. It is possible that absorbed oxygen might claim some of the action of the charcoal.

One further trial was made upon the liquid of a pure culture of yeast in a sugar-salts solution, which had fermented out to "dryness." In this case the amount of iodine required was equivalent of 0·0128 %  $\text{SO}_2$ , which shows perhaps more strikingly the uselessness of the method.

Combined sulphurous acid is estimated by subtracting the amount of "free" from the "total" sulphurous acid; this latter is found by a distillation process which is dealt with below under the heading "methods."

According to Kerp (quoted by Lunge, p. 623) a portion of the  $\text{SO}_2$  added to wines becomes oxidised to sulphuric acid, but the greater part combines with aldehydes, sugar, etc., so firmly that iodine is no longer able to destroy and oxidise the combination. This cannot be said to be true of sound cider, in which the reducing substances aid in sheltering the  $\text{SO}_2$  from oxidation. It may be added that iodine seems to be able to oxidise all the  $\text{SO}_2$  provided that time is allowed.

This definition of "free" acid does not seem to be of great value, and I think that such portion of the acid as might come off with a plain distillation process might be more worthy of the title "free sulphurous acid."

The following two experiments were made in order to see what proportion of the total sulphur dioxide would distil off from sulphited cider without any addition of mineral acid to liberate it.

The results obtained were:—

**Sample A.**

Distilled without any addition gave  $\text{BaSO}_4$  0·0742 g. = 0·02036 g.  $\text{SO}_2$  %.  
Distilled with  $\text{P}_2\text{O}_5$  gave  $\text{BaSO}_4$  0·0809 g. = 0·0222 g.  $\text{SO}_2$  %.

**Sample B.**

Distilled without any addition gave  $\text{BaSO}_4$  0·0961 g. = 0·0527 g.  $\text{SO}_2$  %.  
Distilled with  $\text{P}_2\text{O}_5$  gave  $\text{BaSO}_4$  0·1115 g. = 0·06068 g.  $\text{SO}_2$  %.  
Direct titration with iodine gave equivalent of 0·0224 g.  $\text{SO}_2$  %.

These samples gave contained sulphuric acid ( $\text{SO}_3$ ) 0·0068 and 0·0087 g. per 100 c.c. respectively.

It will be seen that a very large proportion of the  $\text{SO}_2$  was sufficiently free and volatile to pass over without the liberating action of the strong mineral acid; moreover, as will be shown hereafter, the sulphuric acid is within normal limits, so that no considerable amount of oxidation can have occurred. Inasmuch as there was a possibility that some amount of oxidation might have taken place in samples, especially if acetification took place during manufacture, as a routine, I took estimates of the sulphuric, as well as of the sulphurous acid in all the samples which were tested.

It may be concluded that the term "free" sulphurous acid in ciders might well be defined as the amount which is sufficiently free to pass over on distillation when no liberating acid is used.

#### *Methods.*

The simplest mode of revealing the presence of some preservative in cider, is to place a portion of the sample to be tested in a sterilised plugged tube and inoculate it with a pure vigorous culture of cider yeast. If necessary sugar may be added to make the proportion about 3 or 4 per cent. Inasmuch as there may be a poor supply of nitrogenous food in the cider it may be desirable to add a small proportion of an ammonium salt, such as the phosphate, but not more than 2—3 milligrams per 100 c.c. Many samples of cider, which are really free from antiseptic additions, do not require this addition of nitrogenous and phosphatic food and probably it is unnecessary in any case, if thoroughly well-nourished yeast culture is used. It must be remembered that if too much help be given to the yeasts, they will more readily and rapidly overcome small proportions of inhibitory substances. As a routine, I put up two lots, one with the addition of the ammonic salt and the other without. In some two to four days the effervescence of active fermentations should have set in, if it do not the presence of some preservative may be suspected.

In the case of heavily sulphured or more correctly "sulphited" ciders, it will be seen that the yeast does grow to some extent in the form of flocculi and without production of turbidity at first; but after a more or less prolonged interval the sulphites become oxidised to sulphates and the yeasts become enabled to attack the sugar in the usual way. In one case, where a nearly full pint bottle of a sulphited

product was inoculated and plugged with cotton wool, the delay in oxidation, owing to the small amount of air contact, was such that the amount of sugar remained unaltered for three months; eventually the sugar fermented out and all the sulphurous acid had been oxidised to sulphate.

Whilst this fermentation test only indicates the presence of some preservative or other in general, yet when the antiseptic action disappears spontaneously there is a presumption in favour of the employment of sulphite.

The samples were obtained in the open market, sometimes by the kindness of friends. I am also indebted to Mr Barker, Director of the National Fruit and Cider Institute, for two samples made at the Institute.

*Sulphurous acid.* The determination of the "total" sulphurous acid was made as given by Lunge (*op. cit.* p. 621); namely  $\text{CO}_2$  was run through the apparatus, 100 c.c. of the freshly opened sample were run in, and 5 c.c. of syrupy phosphoric acid added. The  $\text{CO}_2$  current was allowed to slacken off till towards the end of the distillation, for the samples usually contained enough to give a fair current. An upright glass worm condenser was used, and led into a "Peligot" (triple bulb U-tube) tube containing the iodine solution; I found it advantageous and economical only to introduce about 10 c.c. of the latter solution at first and then add more from time to time if the colour showed signs of disappearance, thereby a rough idea of the amount of sulphurous acid may be formed and a waste of the iodine solution avoided. The absence of sulphate in the iodine and other reagents was of course determined. Finally the  $\text{SO}_2$  in the distillate was determined gravimetrically as barium sulphate.

In order to avoid the tedious gravimetric process, some attempts were made to titrate the iodised distillate directly with thiosulphate; but it was found that the results were not satisfactory, probably because some bodies are carried over in the distillation which are capable of combining with the iodine. For approximate results Dr Bigelow informs me that this process is sometimes employed in the Department of Agriculture, U.S.A., but errors are considered to arise from volatilisation of part of the iodine.

A mode of attacking the question of the differentiation of "free" and combined acid, which I tried, may be mentioned. This was by attempting fractional precipitation of barium sulphate in the course of direct iodine titrations, with subsequent additions of iodine; two

circumstances, however, made the attempt useless, for the fineness of the precipitate caused too much loss through the filter, and the difficulty of avoiding all air contact during filtration was too great.

*Sulphuric acid.* The determination of the sulphuric acid demands a few remarks for the standard method (*vide Lunge op. cit.* p. 605) leaves something to be desired. The sample, acidified with HCl, is thoroughly boiled and BaCl<sub>2</sub> (about 2 c.c. of 10 % solution) is added; if, then, the precipitate is filtered off, washed and merely incinerated, as is advised, an appreciable loss of BaSO<sub>4</sub> is often entailed because the precipitate comes down rather too fine in the presence of the sugar, etc., of the cider to be well retained by the "baryta" filter paper; it is, however, retained by virtue of organic matters which are precipitated with it. On washing with water, and still more with alcohol, much of this organic precipitate can be removed, but then BaSO<sub>4</sub> in appreciable amount also passes away. On the other hand, when the incinerated precipitate is treated with sulphuric acid to decompose carbonate, there may be a gain of BaSO<sub>4</sub>, if the washing process has not been sufficiently thorough to remove all barium compounds other than the sulphate.

In order to avoid these sources of fallacy the following process was adopted. When a perfectly bright filtrate was obtained, a slight washing with water was given, the filtrate being collected in a clean flask to see that no BaSO<sub>4</sub> was lost. The paper was then allowed to drain and nearly dry spontaneously. It was then cut into three by cuts concentric with the paper. First the point of the cone (containing the greater part of the sulphate) was dabbed carefully into molten fusion mixture (KNO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub>), the next zone was similarly treated; the outer zone was sometimes burnt directly and added to the fusion.

In this way the organic impurities are rapidly oxidised and the sulphate remains uninjured; strictly speaking, the result obtained is the "total sulphur" in the paper and precipitate, but, in the case of yeast-free cider, the margin of inaccuracy is very negligible. If damp, the paper and precipitate gently fizz in the fusion mixture without spluttering or inflaming, if a slight amount of care in dabbing is used; if the paper is quite dry great care must be taken or the paper will blaze and carbonisation will occur, it is best to remove the gas burner whilst the paper is dabbed.

The fusion mass is then taken up with HCl and water, freed from nitrate, the resulting BaSO<sub>4</sub> purified with successive lots of HCl, and finally weighed. I find that some iron compound is always carried down with the barium salt and is not easily removed completely.

*Mode of addition of sulphites.*

The French law lays down that the sulphurous acid shall be pure, and proceed from the combustion of sulphur; further, that where alkaline bisulphites are used they shall be pure and crystalline.

Sulphiting may be performed:—

(1) By the fumes from burning sulphur.

(2) By blowing in  $\text{SO}_2$  from a container filled with the liquefied gas.

(3) By adding sulphites of the alkalis.

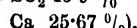
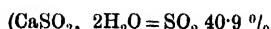
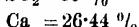
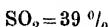
(1) *Fumes of burning sulphur.* The primitive and much practised method of "matching" casks, i.e. burning a strip or "match" of canvas or paper impregnated with sulphur, has already been mentioned<sup>1</sup>.

(2) *Use of liquefied sulphurous acid.* Capsules and cylinders of liquefied sulphurous acid are to be obtained in the market and also arrangements for liberating the desired dose.

Impure solution of the acid can also be cheaply obtained. To what extent either of these products are employed in this country I have no knowledge.

(3) *Use of alkaline bisulphites.* The metabisulphite of potassium seems to be the most favoured salt, especially in France, owing to its high proportion of available  $\text{SO}_2$ , ( $\text{K}_2\text{S}_2\text{O}_5 = 57\cdot6\% \text{ SO}_2$ ). The sodium salts are said not to be much used.

The calcium sulphites are much used by British brewers for adulterating their beer, and it would appear that they are also employed by cider-makers; for, there is a much advertised substance—under the name of "Ham's improved antiferment to preserve the sweets of cider." I obtained a sample of this product and found on analysis, that in its natural air-dried condition it contained:—



According to the instructions, the contents of the packet were to be added to 100 gallons of cider, and if this was not efficacious, a further

<sup>1</sup> According to a legend related by Mr Ozzard at the National Fruit and Cider Institute, Long Ashton, Bristol, an abbot who had trouble with his cider called in a layman to assist him, with promise of great reward in the event of success. Complete success was obtained after a visitation, accompanied by the sight of a cloven hoof, and much smell of sulphur. So the method of sulphuring would not appear to have a very reputable origin!

half-packet should be added. The quantity in the packet weighed 242 grams, which when added to 100 gallons would give the proportion of 53·26 grams per hectolitre, whilst the extra dose would raise it to 79·89 grams per hectolitre. The proportions of SO<sub>2</sub> would then be 212·5 and 318·7 milligrams per litre respectively. These figures may be compared to the present French legal limits—10 grams of alkaline bisulphite per hectolitre and 100 milligrams of total SO<sub>2</sub> per litre.

It is probably not always easy to determine the form in which an addition in the form of sulphite of the alkalis has been made. In the case of the calcium salt (e.g. the above mentioned preservative) there should be a recognisable increase in the calcium when a considerable dose has been used; thus with the dose of 53·26 grams per hectolitre which has been considered in relation to the amount of SO<sub>2</sub>, there would be an increase of about 13·6 grams of calcium per hectolitre or about 13·6 milligrams per 100 c.c. In the case of sample No. 18 (*vide* table, p. 29), which has evidently been heavily sulphited, there was an unusual amount of calcium, viz. 28 mgrms per 100 c.c.; this is three to four times the amount I have hitherto found in ciders or in apple musts, and indeed surpasses the quantity I found, 25·7 mgrms, in the must of the "Holmer" pear. I may add that the beverage in question is advertised as having been made from the apple, although its aroma somewhat suggests that pears have been used in its manufacture. The extra dose of the sulphite preparation (*vide* above, 79·89 grams) would account for about 20 mgrms of Ca per 100 c.c., and this would leave about 8 mgrms for a normal amount of apples and many sorts of pears.

Where the salt used is metabisulphite of potassium, great difficulty would be experienced in determining the source of the SO<sub>2</sub>; this is seen when it is considered that the French allowance of 10 grams per hectolitre only means an increase of about 3·5 milligrams of potassium per 100 c.c. (or the 1907 limit of 20 grams = about 7 mgrms of potassium), both of which fall within the limits given by Kulisch as normal<sup>1</sup>. However, when large doses of the salt have been employed, such as to give an SO<sub>2</sub> proportion of 100 mgrms per litre, there would be an increase of about 60·6 mgrms of potassium, and so on for larger amounts.

Only five of the samples under review have been examined for the determination of the calcium and magnesium, and none for that of

<sup>1</sup> Kulisch gives (*Landwirtschaftliche Jahrbücher*, xix. p. 102):

Apple cider	Calcium	0·0056—0·0132 g. per 100 c.c.
"	Potassium	0·133 — 0·182 "

potassium ; from these analyses it appears that probably the sulphurous acid was introduced as such or as the potash salt.

*Excessive sulphates.* An excessive proportion of sulphates may indicate a sulphited product which has become oxidised from air exposure. Examples of this are afforded by some analyses of the remains of bottles which had been opened for other analyses and then recorked and kept.

Specimen A when obtained in 1907 was tested by the fermentation test and then showed the abnormal flocculent growth of yeast without fermentation ; for the sugar titration showed 3% both originally and again 40 days later ; eventually fermentation set in and the liquor went "dry." Another bottle of the same, kept for a year, and which had been opened for sometime but recorked with an ullage, gave an amount of sulphate equivalent to 201·1 mgrms of  $\text{SO}_3$  per litre, about half of which probably proceeded from an oxidation of added  $\text{SO}_2$ . It was a fancy-named beverage and was thought to have been treated with formaldehyde as well.

Specimen B was the remains of a bottle which had become acetous and gave as sulphates 176·4 mgrms per litre  $\text{SO}_3$ ; probably there were about 68 mgrms of  $\text{SO}_2$  originally.

These figures may be compared with those of Nos. 18 and 21 in the table (p. 29), in both of which the sulphate content is excessive.

At the other end of the scale are the samples Nos 1, 2, 7, and 22 in which the sulphates are extraordinarily low ; it may be noted that these all come from the same county and consequently the peculiarity is probably due to soil and variety of apple.

#### *Analytical details of the samples examined.*

In some of the samples which were examined it was known that no sulphuring whatever had been used. In all of these cases a minute amount of barium sulphate, far too small in amount to weigh, was found in the treated distillate. As the iodine solution by itself, in much greater quantity than that used in these distillations, gave no trace of precipitate with baric chloride, it may be supposed that a minute amount of some organic sulphur compound may be present and volatilised with the vapours in distillation and subsequently oxidised by the iodine.

In the subjoined table all the quantities are given as milligrams per litre. This list would have been considerably extended, but unfortunately many samples were tested with an iodine solution which subsequently

was found to contain sulphate from an impure preparation of potassium iodide; since the amount of iodine solution used had not been measured in each case these had to be rejected, although they showed that the samples were highly sulphited, both by the smallness of the error due to impurity and from the decolourisation of the iodine solution.

#### I. Samples of known sulphuring-free history.

Sample No.	Sulphurous acid ( $\text{SO}_2$ )	Sulphuric acid ( $\text{SO}_3$ )
1 Cider	? minute trace	30·86
2 „	„	10·26
3 „	„	84·36
4 „	„	73·29
5 „	„	113·20
6 Average according to Wagner (German ciders)		90·00

#### II. Samples from open market containing $\text{SO}_2$ .

7 Cider	10·62	17·15
8 „	42·00	60·00
9 „	222·00	68·00
10 Perry	51·30	51·34
11 Cider	194·0	92·3
12 „	123·2	99·9
13 Perry	97·5	80·4
14 Cider	56·2	61·7
15 „	211·4	58·2
16 „	208·2	64·2
17 „	181·2	61·7
18 Fancy name	242·4	198·0
19 Cider	599·0	87·4
20 „	68·2	56·1

#### III. Samples of unknown origin (from bottlers).

21 Cider	? trace	137·8
22 „	„	25·7
23 „	„	106·3

It will be obvious that there is a considerable latitude of idea amongst cider-makers as to the amount of sulphurous acid needed to produce "pure" cider.

The question may well be asked why some ciders (Nos. 1, 2, 3, 4, and 5) can be prepared without sulphites, and that in the absence of salicylic acid or other added preservative, whilst others contain nearly 600 milligrams to the litre. It may be inferred on the one hand that the addition of preservatives is not necessary, and on the other, that negligence or want of control in the extent to which they are added should be regulated. Moreover, many of the sulphited samples are guaranteed to

be free from all additions to the apple juice, whence they were eventually derived ; such statements should not be permissible.

We may now turn seriatim to the various samples which deserve special mention. No. 7, a "pure" product, is evidently free from deliberate additions of sulphite ; it was also free from salicylic acid. Its "purity" apparently included an addition of some form of oak tannin, for it did not give the normal greenish reaction with ferric chloride ; when precipitated with lead acetate (which carries down gallo-tannic acid) the filtrate gave the normal greenish reaction of apple tannin.

Nos. 8, 9, 10 were from the same maker and also free from adulterations. The differences in amount of SO<sub>2</sub> are partly explained by the fact that No. 9 was more sugary than the others, and with regard to the perry I am informed that this maker has the reputation of buying his perry ready made.

Nos. 11, 12 and 13 are the products of one maker, who though working in the same region as the maker of No. 14, uses twice to thrice as much SO<sub>2</sub>.

Nos. 15 to 18 are all products of one firm, which is evidently addicted to the use of the acid in considerable proportions. Further remarks upon No. 18 have already been given, but the high values of both sulphurous and sulphuric acids may again be pointed out.

No. 19 stands out from all the other samples by its huge amount of sulphite. This is the kind of preparation which may help to cause consumers to consider that cider is a beverage which causes gastric discomfort. Several estimations were made from different bottles to confirm the high figure, e.g. Sample "B," p. 22.

No. 21 is an example of an absolutely objectionable concoction, for though no sulphur as sulphite was found, nor indeed salicylic acid, its sweetness was found to depend upon the presence of saccharin. The high figure of the sulphuric acid suggests that originally the sample must have been sulphited and subsequently oxidised.

No. 22 when tested by ether shake was found to be free from salicylic acid, but the residue gave an unusual dirty greenish precipitate with ferric chloride, of unknown nature.

No. 23 was not tested for antiseptics other than SO<sub>2</sub>.

#### *Suggestions and recommendations.*

The suggestions and recommendations which follow may be divided conveniently into two groups ; namely those which affect the addition

of preservatives generally to food products, etc., and those which affect the addition of the special preservative which forms the subject of this paper.

*A. General regulation of preservatives in food products.*

1. Further and more precise regulation is needed in this country at an early date.

2. The presence of added preservatives in food products should only be permitted with declaration of the nature of the preservative. Exceptions of defined substances, such as common salt, should be made.

In declarations such phrases as harmless or unobjectionable additions should not be permitted.

3. Where quantitative distinctions are made, the official method of estimating the quantities should be defined.

4. Where prosecutions are undertaken for illicit additions to food products, not only the vendor and maker of the food product, but also the vendor, maker or importer of the chemical in question should be proceeded against<sup>1</sup>.

5. Manufacturers or importers of food products which contain permissible amounts of permissible chemicals should be required to take out licenses to allow them to use these chemicals, and their works or premises should be registered and liable to visits of inspection by the health authority.

6. Manufacturers or importers of permitted chemicals should also be required to license and register themselves. Further when supplying non-permitted agents, which are capable of use as additions to food products, they should obtain information as to the use to which the said substances are destined.

7. Firms or individuals who trade by supplying analyses or certificates of purity should be compelled to report the presence of added permissible or non-permissible preservatives. And they should be liable to prosecution for neglect or misrepresentation.

*B. The regulation of sulphurous acid and sulphite preservatives in cider and perry.*

1. The addition of sulphurous acid and sulphites to cider and perry needs regulation.

<sup>1</sup> *Vide Durham, Journ. Roy. Inst. Pub. Health, xvi. 1908, p. 293.*

2. The permissible limit of addition of the substances should be expressed as "total sulphur dioxide" obtained by distillation with phosphoric acid.

3. Judging from the practice of some makers, the addition of sulphites or other preservative is unnecessary, and from that of other makers whose products only contain relatively small proportions, the maximum legal limit of "total sulphur dioxide" should be low; and at any rate not exceed 100 milligrams per litre.

4. Ciders and perrys containing more than a "*trace*" of sulphite should be labelled with a declaration to that effect. For practical purposes the word "*trace*" might be defined as "less than 10 (or possibly 20) milligrams of sulphur dioxide per litre."

5. Such terms as "pure," "free from preservatives" and the like should not be permitted for ciders which contain more than a "*trace*" of sulphur dioxide.

6. Cider-makers who desire to use sulphite or other preservatives should be required to take out license and registration.

7. Cider-makers and vendors who are unlicensed and in whose products more than a "*trace*" of sulphur dioxide is found, should be liable to prosecution.

8. Cider-makers or vendors, who are licensed for sulphite additions, but in whose products more than the maximum permitted limit of sulphur dioxide is found should be liable to prosecution.

"ON THE RELATIVE EFFICACY OF THE DOULTON,  
BERKEFELD AND BROWNLOW FILTERS."

By ANDREW WILSON, F.R.S.E.

THE following communication has reached the Editors of the *Journal of Hygiene* with the request that it shall be published<sup>1</sup>:—

DEAR SIR,

In your issue of January 1908, Vol. viii. No. 1, appears an article by Dr William Bulloch and others headed "On the relative efficacy of the Doulton, Berkefeld and Brownlow Filters."

This article states on page 67 that "of 10 'Berkefeld' Filters only one gave a sterile filtrate on the first day, the remaining nine gave contaminated filtrates within 15 minutes, that is to say as soon as the filters were started."

To this statement I, as scientific adviser to the Berkefeld Filter Co., Ltd., 121, Oxford Street, London, W., must take objection on the grounds that the filters were not properly treated by Dr Bulloch, and that the results of his tests are therefore absolutely worthless and must give readers the idea that the "Berkefeld" Filter is not a reliable germ-proof filter.

The treatment of the "Berkefeld" Filter by Dr Bulloch to which I take objection is, that, according to his own statement on page 65 of his article, the filters were sterilised by heating to 120° C. for one hour in the metal cases supplied by the makers.

It is a well-known fact that in consequence of the composition and the mounting of the "Berkefeld" Filtering Cylinders, they do not stand sterilisation in an autoclave at 120° C. The only way effectually to sterilise the cylinder without injuring it is to place it in a vessel with

<sup>1</sup> The reply to this letter follows; see pp. 35—45. ED.

cold or tepid water and to boil it for about one hour. These directions are given in all the lists issued by the Berkefeld Filter Co., Ltd., and I have not the least doubt that by sterilising the filtering cylinders in an autoclave at 120° C. the cylinders or the cement by means of which the cylinders are fixed in the metal mounts have been cracked, so much so that, although invisible to the naked eye, the cracks allowed the free passage of the test organisms.

The same mistake in the sterilisation of the filtering cylinders was made by Dr Kirchner, to whose investigations, which lie as far back as 1891, reference is made by Dr Bulloch. I wish to point out moreover that Dr Kirchner's work has been severely criticised by Professor Gruber, of the Hygienic Institute of the University of Vienna (and now Professor and Director of the Hygienic Institute of the University of Munich), who in a paper published in the *Centralblatt für Bakteriologie und Parasitenkunde*, Vol. xiv. 1893, p. 488, has shown that the results are entirely incorrect.

Having supervised exhaustive experiments with the "Berkefeld" Filtering Cylinder, I have always found that any cylinder properly sterilised, yielded an absolutely sterile filtrate. I take this opportunity of drawing your kind attention to the report to the *British Medical Journal* (No. 1768, Nov. 17th, 1894, and No. 1934, Jan. 26th, 1898) by Dr Sims Woodhead, which may be looked upon as a standard work on the testing of Filters.

As it has now come to my and the Berkefeld Filter Co.'s notice that Messrs Doulton & Co., Ltd., Lambeth, London, S.E., are making use of Dr Bulloch's article for advertising purposes, I consider that the Berkefeld Filter Co., Ltd., is entitled to the publication of this explanation in your paper independently of any action they may be advised to take to restrain the circulation of incorrect statements which tend to depreciate the "Berkefeld" Filter.

Yours faithfully,

(Signed) ANDREW WILSON.

## ON THE TRANSMISSION OF AIR AND MICRO- ORGANISMS THROUGH BERKEFELD FILTERS.

By WILLIAM BULLOCH, M.D.,

*Bacteriologist to the London Hospital,*

AND J. ANDERSON CRAW,

*Research Scholar to the Worshipful Company of Grocers, Hon. Demonstrator  
in Physiology, London Hospital Medical College.*

Plate I and one text figure.

IN several former experiments by Bulloch and Craw (1906), Bulloch, Craw and Atkin (1908), and Craw (1908), we determined the relative efficiency of various filters in keeping back micro-organisms. The filters tested were the Doulton ("white" and "brown"), the Chamberland ("F" and "B"), the Berkefeld, and the Slack and Brownlow. Our methods of experimentation embraced filtration of cultures of microbes in bouillon, and the filtration of tap water under constant and variable pressures. The net result was that all the Slack and Brownlow, and nine out of ten of the Berkefeld filters employed gave contaminated filtrates within 15 minutes of the time the filtration experiment was commenced. Seven out of the ten Doulton "white" filters gave sterile filtrates for at least three days.

As the tap water pressure varied, in a few seconds, from 32·5 lbs. per square inch to zero the test was a severe one, for Craw (1906) had previously shown that even the finest gelatine filters, suggested by C. J. Martin, were very permeable under sudden variations of pressure.

All the filters employed in our experiments were subjected to the same treatment before testing, viz. sterilisation in the autoclave at 120° C. for one hour. The results which we obtained with the Berkefeld filter were so divergent from those in the experiments made for the *Lancet* by Professor Sims Woodhead and Dr Cartwright Wood (1894

and 1898) that before the publication of our paper one of us (W. B.) communicated with Professor Woodhead to ascertain whether he could offer any likely explanation of the difference. He was unable to do so.

It would appear however that after January 1902 some alteration took place in the manufacture of the Berkefeld filter and although the "new cylinder" is described as being much finer in grain and firmer and more uniform in texture than the "old one" it is possible that from a bacteriological point of view it is not so good.

Considerable dissatisfaction appears to have arisen in reference to our conclusions on the Berkefeld filter, for in a letter sent to one of us (B.) and dated "Celle, Prov. Hannover, 28. viii. 08" Dr H. Nordtmeyer, the inventor or originator of the Kieselguhr Filtersystem-Nordtmeyer-Berkefeld, pointed out that he had already (1891) drawn attention to the fact that the "filters being very bad conductors of heat must not be sterilised by steam or dry heat but should be placed in a vessel with cold or tepid water and boiled for about an hour." He added that by autoclaving at 120° C. the cylinders were probably cracked, and that although the cracks were not visible, they would allow the passage of test organisms. He avers that the passage of microbes into the filtrates in Professor Pfuhl's (1903) experiments was referable to this cause. In conclusion Dr Nordtmeyer suggested that his partner should call upon us to "fully explain all the points in question."

To Dr Nordtmeyer's letter an answer was directed by one of us (B.) to the effect that if he had doubts as to the accuracy of our experiments or had criticisms to offer, the recognised procedure for him would be to write to the *Journal of Hygiene* or other scientific journal and that when he had done so we should make a suitable reply. We added that we had dealt with the question entirely from a scientific point of view and had no connection whatsoever with any commercial aspects of the case. A letter from the Managing Director of the Berkefeld Filter Co. Ltd., London, dated Sept. 14, 1908, was answered in like strain.

Early in November 1908 we received from Professor Nuttall a letter enclosing another from Dr Andrew Wilson, which letter is published in this number of the *Journal of Hygiene* (p. 33), and it is as an answer to this letter and that of Dr Nordtmeyer that the following new experiments have been undertaken with the Berkefeld filter.

It may be added at once that our previous conclusions have been completely confirmed although the new tests were carried out under conditions enormously *more* favourable to the Berkefeld filter than our former ones, the pressure being about  $\frac{1}{40}$  of that previously used.

The objection raised by Drs Nordtmeyer and Wilson seems to be that in our former investigations all the filters were sterilised in an autoclave at 120° C., so that there was a possibility that the difference in coefficients of expansion of the nipples or joints and the filter mass might give rise to cracks in the cement used to unite the two parts of the filter. We have therefore investigated a few more Berkefeld filters sterilised in a manner approved by Dr Nordtmeyer, Dr Andrew Wilson and the Berkefeld Filter Co. Ltd. The filters were placed in a clean vessel with cold or tepid water and boiled for an hour. After being boiled the filter was allowed to cool.

In order to ascertain whether there is any evidence that cracks are produced in the filter while being sterilised in the autoclave at 120° C. we applied a method of testing by air pressure, and we subjoin the results which we obtained with the old filters used in our previous experiments (and which had again been autoclaved at 120° C.) and a series of new filters never previously used for any purpose. For the most part these were purchased from Messrs Baird and Tatlock who obtained them from the Berkefeld Filter Co. Ltd., Oxford St., London. A few were obtained from the Army and Navy Stores.

Whether Berkefeld filters are tested in any way before being placed on the market we do not know, but the air pressure test is frequently used in laboratories to determine the existence of cracks, the air coming streaming out as the pressure is applied.

*Technique.* (Fig. 1.) The pressure applied to the filters was obtained by means of an ordinary bicycle pump *P*, connected to a pressure gauge *G* and also to the filter to be tested. The filters were first tested for perfection of joint (position *J*, fig. 1) and subsequently for permeability of wall (position *W*, fig. 1). In the latter case the joint or neck of the filter was maintained at least half an inch above the surface of the water. The pressure was raised to about 8 lbs. per square inch and allowed to fall gradually till leakage of air ceased. The data as regards leakage given below (Table I) refer to the pressures at which the *cessation* of marked air bubbles took place from joint and wall. The reasons for testing the joint first were (*a*) that it allowed transmission of air first and (*b*) that the inverted position (*J*) eliminated the chance that bubbles apparently emanating from the neck were due to a collection of air bubbles from the wall. From Table I it is evident that there is no very striking differences between the unused and used, i.e. sterilised filters, except perhaps that in general the used filters leaked under lower pressures. The worst of all however was Filter No. 1 which transmitted

air freely at the neck at  $\frac{1}{2}$  lb. of pressure. This filter had never been used. In no filter autoclaved or not-autoclaved could any evidence be found of fissure or fracture as judged by the streaming of air from one point. A series of six new Doulton filters tested under the same conditions and at the same time withstood pressures of 10, 11, 10 $\frac{1}{2}$ , 14, 13 $\frac{1}{2}$ , and 13 lbs. air pressure without a single bubble being detected coming through the filter.

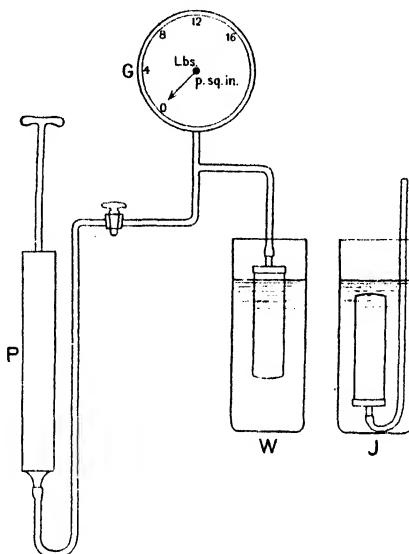


Fig. 1.

#### *Initial Capillary Pressure.*

During the application of the air pressure tests an interesting and important phenomenon was observed on immersing vertically in water at room temperature a new, perfectly dry Berkefeld filter. The filters being connected with the pressure gauge the indicator rose to 5—6 $\frac{1}{4}$  (see fig. 2) lbs. per square inch *although no pressure was applied from the pump*. This phenomenon we can only ascribe to the displacement of the air within the pores of the filter mass by the capillary attraction the latter exerts upon the water in which it is immersed. A similar rise of pressure was indicated on the gauge even when the filter was immersed horizontally, indicating that the rise of pressure is not hydrostatic in nature.

Autoclaved filters on horizontal immersion in water at room temperature gave an initial or auto-pressure of  $2\frac{1}{2}$ —3 lbs. per square inch and in two cases out of three showed leakage at the metal joint without applied pressure, a result which was observed to an equal extent with new filters which had never even been boiled.

TABLE I.

*Examination of Berkefeld Filters for air transmission.*

(Pressure in lbs. per square inch.)

No.	New unused Filters		Used Filters	
	Joint lbs.	Wall lbs.	Joint lbs.	Wall lbs.
1	$\frac{1}{2}$	—	—	—
2	$3\frac{1}{2}$	4	—	—
3	4	4	—	—
4	—	—	2	$3\frac{1}{2}$
5	—	—	2	4
6	—	—	3	4
7	—	—	$2\frac{1}{2}$	3
8	—	—	$4\frac{1}{2}$	6
9	—	—	4	5
10	—	—	1	2
11	5	7	—	—
12	5	5	—	—
13	6	6	—	—
14	$2\frac{1}{2}$	$4\frac{1}{2}$	—	—
15	$3\frac{1}{2}$	$5\frac{1}{4}$	—	—
16	4	6	—	—
17	6	6	—	—
18	6	6	—	—
19	—	—	$4\frac{1}{2}$	$4\frac{1}{2}$
20	—	—	$2\frac{1}{2}$	$2\frac{1}{2}$

Filters 1, 2, 3, 4, 5, 6, 7, 8, 9, 16, 17, 18 had metal tops and were of the kind known as "House Filter F" measuring 5 x 2 in. or 13 x 5 cm.

Filters 10, 11, 12, 13, 14, 15, 19, 20 had porcelain nipples and measured 18 cm. in length and  $2\frac{1}{2}$  cm. diameter, i.e.  $7\frac{1}{4} \times 1$  inch.

*Direct transmission of microbes in water through Berkefeld Filters—new experiments.*

The new filters chosen for experiment were those marked Nos. 13, 14, 16, 17, 18 (Table I).

Nos. 13 and 14 had porcelain ends.

Nos. 16, 17, 18 had metal ends.

TABLE II.

*Re-examination of Berkefeld Filters for air transmission, after steeping 24 hours in water at air temperature.*

No.	New Filters		Used Filters	
	Joint lbs.	Wall lbs.	Joint lbs.	Wall lbs.
1	$\frac{1}{2}$	—	—	—
2	3	3	—	—
3	4	4	—	—
4	—	—	2	$3\frac{1}{2}$
5	—	—	$1\frac{1}{2}$	4
6	—	—	3	4
7	—	—	3	3
8	—	—	$3\frac{3}{4}$	$4\frac{1}{2}$
9	—	—	2	$4\frac{1}{4}$
10	—	—	2	1

The numbers of these filters correspond to those in the previous table and the results give perfectly unbiased information as to air transmission, the notes of Table I not having been compared until the following day.

The filters, with the neck of the metal case and an indiarubber connecting tube with a hooded pipette (as described in our previous communication), were boiled in a large clean vessel for one hour and allowed to cool. With the greatest care the filter was then inserted into the metal case attached to the tap and screwed home. In order that the tests should be less severe than in our previous communications they were carried out in a perfectly quiet laboratory in which the pressure of water was only 9 lbs. per square inch (!) (24.5 inches) in comparison with 32.5 lbs. applied in our former experiments. The supply of water was controlled by a cock. In the course of the experiments the water was plated out on gelatine twice and showed from 100—200 colonies per c.c. After the filtration was started, samples of the filtrate measuring 70 c.c. were run into a peptone salt solution so that the whole was ultimately 1% peptone and  $\frac{1}{2}\%$  salt solution. The samples were then incubated for several days to determine whether they were sterile or not. During the intervals between the collection of the samples the dropping water from the hooded pipette was carefully guarded from contamination by a long sterile glass tube open at the bottom. The results obtained were as follows.

*Examination of Berkefeld Filters for transmission of micro-organisms.*

## FILTER No. 13. (Porcelain nipple.)

Sterilised by boiling in water for 1 hour at 100° C.

Connected with tap at 2.30 p.m. Dec. 16th, 1908. Pressure .87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	3 p.m. (16. xii. 08)	9 c.c. per min.	Growth
II	4 p.m. "	8 "	"
III	12 noon (17. xii. 08)	7 "	"
IV	4 p.m. "	6 "	"
V	11 a.m. (18. xii. 08)	5 "	"

Result: Filtrate contained bacteria within  $\frac{1}{2}$  an hour.

## FILTER No. 14. (Porcelain nipple.)

Sterilised by boiling in water for 1 hour at 100° C.

Connected with tap at 2.10 p.m. (18. xii. 08). Pressure .87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.15 p.m. (18. xii. 08)	16 c.c. per min.	Sterile
II	5.50 p.m. "	10 "	Growth
III	11.30 p.m. (14. xii. 08)	10 "	"
IV	4 p.m. "	6 "	"
V	11 a.m. (15. xii. 08)	5 "	"

Result: Filtrate contained bacteria within 3 hours.

## FILTER No. 16. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.

Connected with tap at 2.30 p.m. (7. xii. 08). Pressure .87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.40 p.m. (7. xii. 08)	30 c.c. per min.	Growth
I <sub>A</sub>	2.50 p.m. "	30 "	"
II	3.30 p.m. "	28 "	"
III	5 p.m. "	27 "	"
IV	2 p.m. (8. xii. 08)	24 "	"

Result: Filtrate contained bacteria within 10 and 20 minutes.

## FILTER No. 17. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.

Connected with tap at 12.10 p.m. (2. xii. 08). Pressure .87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	12.15 p.m. (2. xii. 08)	30 c.c. per min.	Growth
II	12.25 p.m. "	30 "	"
III	1 p.m. "	29 "	"
IV	2 p.m. "	30 "	"
V	4 p.m. "	29 "	"

Filter stopped over night, started 12.30, 3. xii. 08.

VI	12.30 p.m. (3. xii. 08)	29 c.c. per min.	Growth
VII	2 p.m. "	29 "	"
VIII	3.30 p.m. "	29 "	"
IX	12 noon (4. xii. 08)	24 "	"
X	2.40 p.m. "	23 "	"

Result: Filtrate contained bacteria within 5 minutes.

*Berkefeld Filters*

## FILTER No. 18. (Metal end.)

Sterilised by boiling in water for 1 hour at 100° C.

Connected with tap at 2.20 p.m. (10. xii. 08). Pressure .87 lbs. per sq. in.

Sample	Time and date	Rate of filtration	Result
I	2.32 p.m. (10. xii. 08)	26 c.c. per min.	Sterile
II	5 p.m. "	27 "	Growth
III	5 p.m. (11. xii. 08)	17 "	"
IV	12 noon (12. xii. 08)	15 "	"

Result: Filtrate contained bacteria within 2½ hours.

All these five new filters showed contaminated filtrates between 5 minutes and 3 hours of the time filtration was started. These results although carried out under conditions extraordinarily favourable to the Berkefeld bougie were so disastrous to its reputation as a filter that we decided to carry out a still more lenient test—one that we may describe as autofiltration. Under the term "initial capillary pressure" we have already described (p. 38) the peculiar fact that when a dry Berkefeld filter is immersed vertically in water a pressure of 5—6½ lbs. may be registered on the indicator of the gauge. This led us to observe what is going on inside the filter during the period of immersion by sawing off the metal or porcelain end. On dipping one of these cut filters into water containing a solution of acid fuchsin a large quantity of the fuchsin appeared in the lumen of the filter within a few minutes, and it suggested itself to us that possibly bacteria might come through by "autofiltration" alone. Two filters were chosen, one No. 16 used in the above experiments (p. 41) and No. 15 which had never been used. The outside of No. 16 was carefully brushed and both filters were then dried in a hot air chamber at 54° C. At the end of 24 hours both filters held in vertical position on stands were slowly and carefully lowered into a vessel of water which had been previously inoculated with a culture of *Bacillus prodigiosus* and incubated at 25° C. for two days. The utmost care was taken to prevent the water coming into contact with the cement of the joint which stood nearly 1 inch above the level of the water. The hydrostatic pressure amounted to only 9 cm. of water (0·12 lbs. per sq. inch for the base of the filter and a mean pressure of .06 lbs. per sq. inch). By means of a series of long, sterile Pasteur pipettes the water which passed into the lumen of the filter was removed and inseminated into bouillon and on agar with the following result.

Sample	Time after starting	Volume of filtrate	Result
Filter 16.	1	6 mins. 1 c.c.	Sterile
	2	7 " 1 "	"
	3	8 " 1 "	"
	4	10 " 6 "	"
	5	11 " 2 "	"

After the removal of the 5th sample 5 c.c. of bouillon was introduced by means of a sterile pipette into the lumen of the filter, the metal end being well flamed by a bunsen, a sterile cotton wool plug was placed in the opening and the filter left in the prodigiosus water over night. Next day a sample of the contents of the filter lumen was plated out on agar and showed numerous colonies of *B. prodigiosus*. Thus while we were unable to demonstrate the *B. prodigiosus* directly in samples as large as 6 c.c. the microbe had apparently passed through and we demonstrated this indirectly. In order to get a large initial volume of filtrate for inoculation we allowed Filter 15—the new filter—to stand in the prodigiosus water for 20 minutes and at the end of this time we abstracted about 35 c.c. from the interior of the filter, inseminating the whole in peptone water. After incubation for three days abundant growth of prodigiosus was proved by plating on agar. Thus the microbe had passed from the water through the wall of a perfectly new Berkefeld filter.

#### *On Leakage at Joint of Filters.*

An important point, which seems to have been generally overlooked, is the relative efficiency of the joint and the wall of filters. The large Berkefeld filter has a metal neck or socket into which the Kieselguhr mass is cemented. It would be strange if a composite filter of this description could with certainty withstand boiling when one considers the relative coefficients of expansion of the majority of the metals and the majority of siliceous materials. It appears to us that the metal socket consists of brass which has been nickel-plated. Now the linear coefficients of expansion of these substances on heating from the freezing point to the boiling point of water are as follows:—brass expands 188 %, siliceous materials 06—1 % of their lengths, that is to say, the metal expands from 1·88 to 3·13 times more than the siliceous material. The superficial or planar and the cubic coefficients show a greater divergence. It seems to us improbable that any cement has yet been discovered which could resist the strain thus implied if it were used to unite metal and siliceous matter. Any filter therefore of this composite character, such as for example the large Berkefeld bougie, may show leakage at the neck or joint after heating in boiling water, and might possibly show such leakage before any attempt has been made at sterilisation, owing to variations in room temperature during storage. Whether this is the true explanation or not, the fact remains that in

the great majority of Berkefeld filters tested by us the neck or joint leaked at much lower pressures than the wall. On the other hand the Doulton filters consisting of porcelain nipple and porcelain wall did not show this effect.

#### CONCLUSIONS.

##### *Transmission of Air.*

1. The passage of air through the joint and wall of the Berkefeld filter does not seem to be materially affected by (a) boiling in water, or (b) autoclaving at 120° C., or (c) soaking new and used filters in water for 24 hours.

2. These filters leaked, in general, at the neck or joint at lower pressures than at the wall, the joint from  $\frac{1}{2}$  to 6, the wall from 4 to 7 lbs. per square inch with new filters. Used filters showed leakage at the joint from 1 to  $4\frac{1}{2}$ , and wall 2 to 6 lbs. per square inch.

3. Simple immersion of a dry Berkefeld filter may give rise to a pressure of over 6 lbs. per square inch and this caused immediate leaking at the joint. With autoclaved, comparatively moist filters a pressure of 3 lbs. per square inch was obtained with leakage at joint. We have thus an auto-transmission of air.

4. At the pressure used in our experiments, viz. 0·9 lbs. per square inch, the large Berkefeld filters gave a yield of filtrate approximating to 0·4 gallons per hour. This is an exceptionally lenient test for a filter which the Berkefeld Company consider should give about 6 gallons an hour, i.e. 15 times as much.

##### *Transmission of Micro-organisms.*

5. Of two Berkefeld filters with porcelain nipples, one gave a contaminated filtrate from ordinary London tap water immediately, the other likewise after 3 hrs. 40 mins. These were new filters which had not been autoclaved but merely boiled one hour.

6. Of three Berkefeld filters with metal nipples, two gave immediate contamination with tap water and the third after 2 hrs. 40 mins. These filters were likewise new and had only been boiled one hour.

7. Two dried Berkefeld filters, one with metal and one with porcelain ends, on immersion of a portion of their walls in a water culture of *B. prodigiosus* allowed this organism to pass into the interior of the filters.



JOURNAL OF HYGIENE, VOL. IX. NO. 1

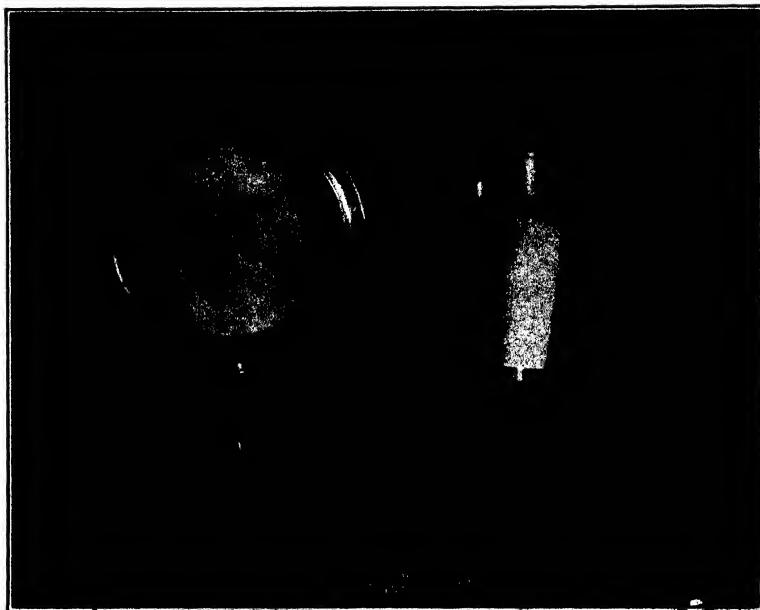


Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



8. These results are entirely confirmatory of our former work and effectually dispose of the objections raised in the private communications received from Messrs Nordtmeyer and Andrew Wilson.

### REFERENCES.

- BULLOCH and CRAW (1906). *Journ. of Hygiene*, vi. p. 409.  
 BULLOCH, CRAW and ATKIN (1908). *Journ. of Hygiene*, viii. p. 63.  
 CRAW (1906). *Proc. Roy. Soc. London*, 77 B. p. 311.  
 NORDTMEYER (1891). *Zeitschr. f. Hygiene*, x. p. 145.  
 PFUHL (1903). *Festschr. z. 60sten Geburtstag von R. Koch*, Jena, p. 75.  
 WOODHEAD and WOOD (1894). *Brit. Med. Journ.* ii. pp. 1053, 1118, 1182, 1375,  
     1486.  
 —— (1898). *Ibid.* i. p. 261.

### EXPLANATION OF PLATE I.

- Fig. 1 shows an initial capillary pressure of 6 lbs. per square inch by simple immersion of a new Berkefeld filter without applied pressure.  
 Fig. 2 shows bubbles of air accumulating on the surface of water in which is immersed a new Berkefeld filter under an air pressure of  $5\frac{1}{2}$  lbs.  
 Fig. 3 shows the same filter (as in Fig. 2) under an air pressure of 6 lbs. per square inch. The extra  $\frac{1}{2}$  lb. of pressure causing streaming of air bubbles from every pore of the wall, as is indicated in the photograph by the fact that the water above the joint of the filter is opalescent.  
 Fig. 4. Doulton white filter under same conditions as Figs. 2 and 3, with gauge showing pressure of  $13\frac{1}{2}$  lbs. per square inch. No bubbles : water perfectly transparent.

## ON THE DANYSZ EFFECT WITH REFERENCE TO THE TOXIN-ANTITOXIN REACTION.

By J. A. CRAW,

*Grocers' Company Research Scholar, Hon. Demonstrator in Physiology  
London Hospital Medical College.*

One Figure.

SVANTE ARRHENIUS (1908) has taken exception to my criticism (1907) of his interpretation of the Danysz Effect with reference to the Toxin-Antitoxin reaction. In this paper he has given an interesting but rather far-fetched analogy to the Danysz Effect, which is, according to him, purely chemical in character and subject to the laws of chemical mass action, and here marks in italics that "*the opponents of the use of these laws have still given no explanation at all of the Danysz Effect, especially of the experiments cited in our memoir.*"

In this communication I shall, after replying to the objections advanced by Arrhenius, endeavour to give the missing explanation based on the phenomena of adsorption and determine in how far this more physical view is compatible with my own experimental work and with that of Madsen and Walbum in Madsen and Arrhenius's Memoir (1906). In the first place it is necessary to indicate the fundamental significance of the Danysz Effect in the interpretation of the nature of the toxin-antitoxin reaction.

At present the interaction of bacterial toxins with their specific antitoxins is regarded as being practically independent of biological influences and either of a chemical or a physical nature. Of the many views advanced the most definitely crystallised are the purely chemical conceptions of Ehrlich and of Arrhenius and Madsen.

Ehrlich considers the toxic fluids to be mixtures of several toxic chemical substances having different degrees of affinity for the corresponding antitoxins, and he regards the reaction of toxin and antitoxin

as the successive neutralisation in steplike manner of the different toxic constituents, analogous to the neutralisation of a mixture of acids by a base.

Arrhenius and Madsen agree with Ehrlich in regarding the toxins as possibly complex in nature and their neutralisation by antitoxin as similar to the known reactions of acids on bases, but differ from him in the interpretation of the data bearing on the course of the neutralisation. They consider that experimental work so far shows only the neutralisation of one toxic substance, viz. that toxic constituent of the toxic fluid with the greatest affinity for the antitoxin, and that the course of this neutralisation with varying masses of toxic and antitoxic fluids is similar to that obtaining in the neutralisation of a single weak acid by a weak base, e.g. the interaction of boracic acid and ammonia in aqueous solution.

They find that the law of chemical mass action enunciated by Guldberg and Waage holds for the reaction between this most toxic constituent and antitoxin, and, further, that similar application of this fundamental relation between chemically active masses is justifiable in numerous reactions in Immunity. Arrhenius and Madsen do not hesitate to push the analogy to its limit and conclude that the stoichiometric relations existing between the ultimate particles or "molecules" of toxin and of antitoxin are disclosed by their experiments. Likewise the absorption relations of agglutinin reveal, according to Arrhenius, the dissociation of the agglutinin "molecule" within the absorbent micro-organisms. Arrhenius has found that a certain formula which he maintains is based on the law of chemical mass action expresses with close approximation the quantitative mass relations in thirty-five different cases taken from different departments of Immunity (*Immuno-chemistry* by S. Arrhenius (1907), The Macmillan Co., New York) and considers his fundamental assumptions and methods of calculation to be entirely justified by this general agreement.

The experiments of Danysz (1902) on the neutralisation of ricin and of diphtheria toxin by their corresponding antibodies introduce, however, a new factor necessitating considerable modification of both the theory of Ehrlich and that of Arrhenius. Danysz found that when the toxin was added to the antitoxin in two fractions, a considerable time being allowed to elapse between the additions, the resultant mixture contained a much larger amount of free toxin than in the case when the total quantity of toxin was added to the antitoxin at once. This abnormally high toxicity of a toxin on its fractional addition to

an antitoxin is known as the "Danysz Effect." The "Effect" was explained by von Dungern (1904) as due to the neutralising effect of a new constituent of the toxin, viz. "epitoxonoid," a view subsequently endorsed by Sachs (1904) and Ehrlich.

Arrhenius (1908) attributes the Danysz Effect to a secondary neutralisation process similar in some respects to hydrolysis which proceeds at a lower rate than the primary neutralisation of toxin by antitoxin corresponding to the single acid-alkali relation.

Thus the toxin may be regarded as behaving similarly to chloracetic acid when the latter is added to sodium hydrate, and the antitoxin acts in an analogous manner to the alkali. In experiments of the Danysz Effect type the *first fraction of toxin* or acid is rapidly neutralised by the antitoxin or alkali which is present in excess. If this mixture be allowed to stand a secondary reaction takes place between the excess of antitoxin and the neutralised toxin which becomes more complete with increasing time of contact and corresponds to the pseudo-hydrolytic action studied by Schwab (1883) of the excess of sodium hydrate on the product of the primary neutralisation, viz. the reaction of sodium chloracetate with the alkali to produce sodium glycollate and sodium chloride. During this secondary pseudo-hydrolytic process the excess of antitoxin or of alkali, respectively, decreases considerably and, consequently, on the addition of the second fraction of toxin or acid the antitoxin or alkali is no longer present in sufficient quantity to neutralise the toxin or acid and the resultant mixtures are toxic and acid respectively. When the *total quantities of toxin* or acid respectively are added at once to the antitoxin or alkali the secondary hydrolytic process is negligible and all the antitoxin or alkali is available for the primary neutralisation which is consequently more complete.

On the other hand Nernst (1904) and Craw (1905, I), (1905, III), (1907, VI) have pointed out many difficulties in the application of the laws of chemical mass action to the reactions in Immunity in the manner adopted by Arrhenius; and Bordet (1903), Lauststeiner (1903), Craw (13, II, 1904), Biltz (1904), etc. have indicated that similar quantitative relations hold for the staining act. Thus in the toxin-antitoxin reaction the toxin corresponds to the dye and the antitoxin to the fabric or tissue stained. The free toxin in equilibrium with the bound toxin is found to obey roughly the same general law as holds for the free dye and dye fixed by the tissue, a law closely allied to, if not identical with, that holding for the extraction of substances

from solution by porous bodies such as charcoal and classed under the general heading of adsorption.

Further I found (1905, 1) that the Danysz Effect has its counterpart in the phenomena of staining, a result which has since been confirmed by Bayliss (1906). The general formula hitherto used to express adsorption relations, viz.  $C_2 = KC_1^n$ , where  $C_2$  is the concentration of adsorbed substance in the adsorbent material,  $C_1$  the concentration of the same substance existing in aqueous solution, and  $K$  and  $n$  constants dependent upon the nature of the aqueous solvent, the adsorbed material and the temperature, is admittedly an empirical relation and no satisfactory chemical or physical explanation of the meaning of these constants has hitherto been advanced. There being no physical reason why such a relation should hold for adsorption in general I can only attribute the prominence given to the formula as due to the impression that most curves roughly resembling hyperbolas obey the law  $Yx^m = \text{constant}$ . This however is not necessarily so and the simplest test of the formula by plotting  $\log C_2$  against  $\log C_1$  shows that in *purely adsorption phenomena* the straight line relationship is but a rough approximation.

I have verified this in the cases of the adsorption of iodine by charcoal and of congo red by filter paper from aqueous solutions. To obtain constancy of the ratio  $\frac{C_2}{C_1^n}$  a tendency of  $n$  to approach unity as the concentration of free iodine or congo red increases must be assumed. This tendency seems in fact to be the general rule in adsorption phenomena as the work of Hoitsema (1895), Ramsay, Mond and Shields, and Travers (1906) on the adsorption of hydrogen by palladium, of oxygen by palladium, of hydrogen by platinum and of hydrogen and carbon dioxide by carbon definitely shows. Here also the general adsorption formula given above holds throughout a certain range of gas pressures but the value of  $n$  is variable with the temperature, approaching more towards unity at the higher temperatures and particularly so for the higher pressures at these higher temperatures.

If the value of  $n$  in the adsorption formula be similarly modified for systems containing toxin and antitoxin, especially for the higher concentration of toxin, we obtain an empirical formula which reproduces the experimental data exactly, and the same holds for all of the cases examined by Arrhenius and Madsen which show approximation to their general formula. The latter general formula however does not exactly reproduce the observations and can most easily be brought into con-

sonance with the experimental data by assuming that the power to which the fixed or bound toxin is raised is not constant and that the formula giving the exact reproduction of the observed data is not

$$\frac{\text{Free Toxin}}{\text{Concentration}} \times \frac{\text{Free Antitoxin}}{\text{Concentration}} = K \left\{ \frac{\text{Bound}}{\text{Antitoxin}} \right\}^2,$$

but

$$\frac{\text{Free Toxin}}{\text{Concentration}} \times \frac{\text{Free Antitoxin}}{\text{Concentration}} = K \left\{ \frac{\text{Bound}}{\text{Antitoxin}} \right\}^n,$$

where  $n$  is *nearly* equal to 2.

As formerly pointed out the antitoxin concentration is determined empirically and introduces a constant  $p$  indicating its equivalence to toxin. We have thus two constants  $p$  and  $K$  and a variable  $n$  by means of which an exact reproduction of the data may be obtained.

Since both the adsorption formula and the chemical mass action formula of Arrhenius do not exactly agree with the observation, but can both be modified by a slight change of the power to which the fixed toxin is raised in such a manner as to give in each case perfect agreement with the experimental data, they are of equal value as empirical formulae. The whole problem resolves itself into the interpretation of the physical and chemical meaning of the power  $n$  in the two equations and of  $p$  and  $K$  of the chemical mass action and  $K$  of the adsorption views.

It is therefore necessary to trace as far as possible (i) the significance of the adsorption formula regarded from a purely chemical standpoint and likewise (ii) the meaning of the chemical mass action law from an adsorption point of view.

### *1. On the meaning of the adsorption formula from a purely chemical standpoint.*

This problem will be pursued further on a future occasion but a preliminary view can be advanced giving the chief features. The adsorbing substance, *e.g.* amorphous carbon, spongy platinum, palladium, and colloidal "gels" including living organisms, etc., behave as, in the simplest case, a single phase. The medium containing the substance about to undergo adsorption is in general either gaseous or aqueous, and forms a second phase. The substance to be adsorbed, which may be gaseous, crystalloidal, or colloidal, forms part of the second phase and is, it is presumed, subject to the laws of partition holding for the distribution of gas or crystalloidal substance between two phases. A

few examples of this fundamental law, in its simplest form, are the partition of oxygen, nitrogen and hydrogen between gaseous medium and water. The partition law is here known as Henry's law and states that the pressure  $p_1$  of free gas at constant temperature is proportional to the gas-like or osmotic pressure  $p_2$  of the dissolved gas, so that  $P_1 = KP_2$ , where  $K$  is the proportionality constant.

Now the gaseous and osmotic pressures are inversely as the gaseous concentration  $C_1$  or osmotic concentration  $C_2$  of the molecules of the substance present in the two phases, from which we obtain  $C_2 = KC_1$ . More easily condensable gases do not however obey this simple law exactly, but a very close approximation is obtained to constancy when the ratio  $\frac{C_2}{C_1^n}$  is calculated where  $n$  is a second constant. This is, in fact, the formula used in tracing adsorption relations, and to obtain perfect agreement with the observed data,  $n$  has to be slightly modified when the range of concentration  $C_1$  is great, in a similar manner to the modification required to give exact agreement with adsorption data.

But, in this case, a definite significance can be given to the value of  $n$ , for it represents the ratio existing between the number of molecules in the gaseous state and the number in the dissolved state to which the former give rise on solution, thus  $n = \frac{2}{3}$  means that two gaseous molecules on solution give rise to three. The variability of  $n$  which occurs with variation of concentration seems to me to be a necessary consequence of the molecular view. When it is considered that Boyle's law does not hold exactly for the relations between pressure and volume with easily condensable gases and as a similar deviation from the analogous osmotic pressure and volume relations exists which is not of the same magnitude as the gaseous deviation, the ratio  $n$  for high concentrations in both phases must differ from the ratio  $n$  at low concentrations.

The value of  $n$  in many cases of solution, of occlusion and of adsorption seems to me however to be scarcely compatible with the purely chemical view that we are dealing with relations existing between molecules in two phases, e.g. Hoitsema (1895) from his experiments on the solution of hydrogen gas in palladium is forced to conclude that the gas molecule  $H_2$  on solution is dissociated into  $H$  atoms but that with increasing concentration of hydrogen the dissolved matter associates more and more and the solution contains more and more complexes  $H_2$ , i.e. of the same molecular magnitude as the gaseous molecule. This is however more clearly shown by the very exact figures of Travers (*loc. cit.* 1906)

who shows that hydrogen dissolved in amorphous carbon at -190 deg. C. must be dissociated into fragments of an atom. Ramsay previously had shown that sodium partitioned itself between the gaseous phase and a mercury phase in such a manner that the dissolved sodium ought to be in a subatomic state. Travers has further indicated that carbon dioxide in solution in carbon at 0 deg. C. behaves like hydrogen at -190 deg. C.—the carbon dioxide must therefore be dissociated into a submolecular state.

Similar relations hold for the partition law when a substance is distributed between two solvents. Thus Nernst (1891) found that when benzoic acid was partitioned between water and benzene the value of  $n$  indicated that in benzene the molecules were double the magnitude of those dissolved in water. But when we attempt to interpret the value of  $n$  for adsorption when the adsorbing substance is placed in an aqueous solution of the substance to be adsorbed, we are met with similar difficulties to those mentioned above for occlusion. Thus the partitioning of iodine between an aqueous solution and amorphous carbon gives the general relation  $C_2 = KC_1^n$ , where  $n$  is nearly =  $\frac{1}{4}$ . This indicates that one molecule of the iodine dissolved in water dissociates into four parts in the carbon—or that it exists in the subatomic state. Similarly in washing potassium iodide out of charcoal I find  $n$  less than unity. But the potassium iodine, KI in aqueous solution is fully dissociated into the ions  $\overset{+}{K}$  and  $\overset{-}{I}$ , consequently in carbon the  $\overset{+}{K}$  ion must have dissociated and likewise the  $\overset{-}{I}$  ion. Similar conclusions must be drawn for the equilibria between salt solutions and paper, and dissociations of simple substances must be assumed where no such dissociation appears to be possible from the present standpoint of physical chemistry. It seems to me therefore that the interpretation of the adsorption formula as showing the relations existing between chemical molecules in the free and adsorbed states leads to highly improbable views of the structure of the substances adsorbed. This being so for substances of known molecular weight it seems to me to be still more doubtful to interpret the partition law as showing molecular relations in the case of substances of the nature of colloids etc., the molecular weights of which are unknown and the degree and nature of the aqueous solutions of which are certainly to a great extent different from crystalloidal solution. Amongst these substances of unknown state of solution or of colloidal nature we must include the great majority of the substances dealt with in Immunity and particularly the toxins,

antitoxins, agglutinins and agglutinable substances. Thus Arrhenius's statement that agglutinin on being partitioned between a saline medium and bacteria undergoes dissociation, two molecules of free agglutinin giving rise to three molecules of adsorbed agglutinin for  $n = \frac{2}{3}$ , rests on the slenderest of bases, viz. on the chemical interpretation of the value of  $n$ .

## 2. *The meaning of the chemical mass action law from an adsorption point of view.*

Arrhenius's general law for toxin and antitoxin interaction mentioned above is that first found by Arrhenius and Madsen (1902) for tetanolysin and its antilysin.

With a constant quantity of toxin and different quantities of antitoxin the equilibria attained were well represented by an equation showing that one molecule of toxin reacted with one molecule of antitoxin to produce two molecules of the compound toxin-antitoxin. The chemical mass law representing this interaction is

$$\frac{\text{Free Toxin}}{\text{vol.}} \times \frac{\text{Free Antitoxin}}{\text{vol.}} = \frac{K (\text{Combined Toxin-Antitoxin})^2}{\text{vol.}},$$

or the concentration of free toxin multiplied by the concentration of free antitoxin is proportional to the square of the toxin-antitoxin compound concentration. This formula is however only apparently simple. Let us inquire how the various factors are determined. The total toxin and total antitoxin are directly measured in cubic centimetres of fluid containing constant quantities of the reacting substances (of unknown molecular concentration). The third experimental datum is the determination of the toxin left free in each mixture. We now proceed to deductions: (1) the concentration of the bound toxin is assumed to be equal to the difference between the concentration of the added toxin and the toxin left free. This, however, entirely overlooks the probability that the antitoxin is either a colloid or bound to a colloid and that *the bound toxin would be concentrated in the antitoxin and not distributed throughout the whole aqueous medium* in the same way as the free toxin.

(2) The concentration of bound antitoxin is arbitrarily made equal to the bound toxin. But all evidence tends to show that no such simple stoichiometric relation persists even in the case of molecular compounds when the components are varied in relative concentration.

(3) The free antitoxin concentration is then taken as the difference

between the concentration of antitoxin added and the bound antitoxin calculated.

(4) The total antitoxin is further not taken as the number of cubic centimetres of antitoxic fluid added in the experiments but is only assumed to be proportional to the latter and the proportionality factor  $p$  is interpolated from the whole series of equations corresponding to different amounts of antitoxin. From these we find that  $N$  c.cs. of antitoxic fluid correspond to  $Np$  c.cs. of toxin. The equation of Arrhenius thus gives only the relation

$$\text{Free toxin} \times (\text{Toxic capacity of antitoxin} - \text{Bound toxin}) = K (\text{Bound toxin})^2,$$

$$\text{or Free toxin} \times \text{Toxic capacity of free antitoxin} = K (\text{Bound toxin})^2.$$

This we may transpose into

$$\frac{\text{Free toxin}}{\text{Bound toxin}} = K \frac{\text{Bound toxin}}{\text{Toxic capacity of free antitoxin}},$$

or in other words the ratio between free and bound toxin is proportional to the ratio between the bound toxin and the amount of toxin which the antitoxin is still capable of taking up. But the bound toxin is the amount that could be taken up by the aqueous fluid to give the original concentration of free toxin. Further the toxic capacity of the free antitoxin is the amount of toxin which could be taken up by the antitoxin to give the original concentration of bound toxin when a very small amount of antitoxin was added to the original concentration of toxin.

So that we obtain

$$\frac{\text{Free toxin}}{\text{Bound toxin}} = K \frac{\text{Toxic capacity of the free water}}{\text{Toxic capacity of the free antitoxin}}.$$

This may be made clear by a diagram based on Arrhenius's and Madsen's figures (see p. 55).

By mere inspection it is evident that at  $A$  the ratio

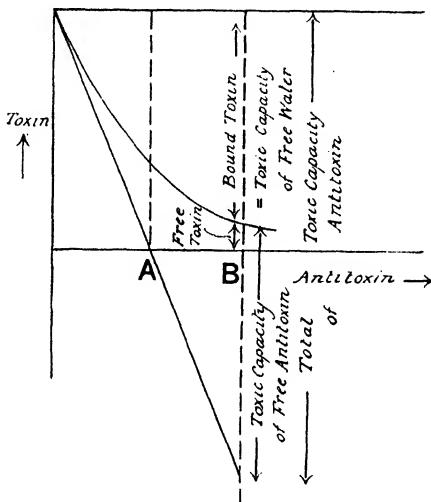
$$\frac{\text{Free toxin}}{\text{Bound toxin}} = \text{about } \frac{1}{3},$$

whereas the ratio between the toxic capacity of the free water and the toxic capacity of the free antitoxin is of course  $\frac{3}{1}$ , we have then

$\frac{1}{3} = K \frac{3}{1}$  or  $K = \frac{1}{9} = 0.11$ . At  $B$   $\frac{\text{Free toxin}}{\text{Bound toxin}} = \frac{1}{11}$ , the other ratio is  $\frac{1}{11}$ , hence  $\frac{1}{11} = K \frac{1}{11}$  or  $K = 0.1$ .

What then is the meaning of this simplified view of Arrhenius and Madsen's highly prized formula? In order to elucidate this important matter we must inquire into the significance of the ratio

$$\frac{\text{Toxic capacity of free water}}{\text{Toxic capacity of free antitoxin}}.$$



Both the numerator and the denominator are dependent upon the initial concentration of toxin in the toxic fluid or, more correctly, upon the absolute amount of toxin, which was the same throughout the series. Had the total quantity of toxin in the toxic fluid used in each individual case throughout the series been greater the saturation of the lower quantities of antitoxin would have been greater and the maximal capacity of the antitoxin for toxin would also have been greater. As a consequence of this the "toxic capacity of free water" would have been greater and likewise the toxic capacity of free antitoxin.

It follows from this that the maximum toxic capacity of an indefinitely small quantity of added antitoxin is equal to the total quantity of toxin used in the experiments in question. Likewise the maximum toxic capacity of the constant quantity of water is equal to the same total quantity of toxin.

The above relationship seems to me to be merely another form of the partition law and would hold equally well for a gas in a closed space to which water was added in small quantities. Thus for ammonia gas and

water in a closed space the relation probably holds that the ratio of free ammonia gas to dissolved ammonia  $\text{NH}_3$ , Aq is proportional to the ratio of the free space and the free water. Here equally the free space represents the difference between the initial concentration of ammonia gas and the concentration after adding a definite small quantity of water. Likewise the free water is merely a term for the difference between the concentration of ammonia in the first exceedingly small fraction of added water and the concentration in any subsequent fraction. Arbitrary constants being used in a similar manner to that adopted by Arrhenius we should arrive at the conclusion that one molecule of ammonia gas combines with one molecule of water to give two molecules of ammonia-water compounds. So far as I have investigated the figures especially for chlorine and water, there seem to be grounds for thinking that some general relation of the type assumed by Arrhenius holds in the majority of solution and adsorption phenomena as a mere corollary of the partition law and without significance as regards the interaction of molecules. In other words Arrhenius's equation may well be regarded as an adsorption interpolation formula once it has been deprived of its *molecular* significance.

#### *Physical interpretation of the Danysz Effect.*

Although the above seems to me to rob Arrhenius's criticism of my objections to his view of the Danysz Effect of its foundations, I hope to furnish shortly a detailed criticism of many minor points he has raised.

I have been led to a more physical explanation of the Danysz Effect as the result of my work during the past year. The study of all kinds of adsorption phenomena seems to me to be most likely to throw light on the subject. With this object in view I have investigated the Danysz Effect in the process of staining, I found that using congo red as the analogue of toxin and filter paper as that of antitoxin, the amount of dye removed from the solution depended upon the manner of addition to, and the distribution of, the paper throughout the dye fluid. The nature of the filter paper influenced to a great extent the amount of dye removed from a given solution and filter papers with a high ash in general removed more congo red than low ash papers. Further, filter paper from which the ash had been removed to a considerable extent showed a decreasing power of adsorbing congo red with decreasing ash content. With papers of low ash slight quantities of sodium and calcium chlorides which had no

precipitating action on the dye greatly increased the adsorption. These facts lead me to the view that the adsorptive power of colloidal substances such as cellulose, globulin, etc. and possibly antitoxins (by analogy) is largely conditioned by their salt content and probably by the specific nature of the adsorbed salts. It is at present too early to do more than indicate that the specificity and high adsorbing power of antitoxin and

*Adsorption of Congo Red by Paper.*

P	.014	.012	.010	.008	.006	.004	.002
A	30	20	7.5	3	2	2	2
B	21	17.5	10	4.5	3	1.75	1.5
C	20	17.5	14	10	3.5	2	2
D	15	13	10	4.5	4	1.75	1.5

P, represents the absolute percentage of Congo Red in the seven solutions used.

A, gives the relative percentage of Congo Red left free with paper moistened by 10 % alcohol.

B, with unwetted paper.

C, with paper wetted with absolute alcohol.

D, with paper moistened with distilled water.

bacteria for certain toxins and agglutinins may depend to a very great extent upon the nature and ratio of the concentrations of the salts contained. The relation existing between free dye and adsorbed dye when dye fluids of different concentrations are brought into contact with moist filter paper is expressed approximately by the formula  $C_2 = KC_1^n$  mentioned above and the deviations are in the sense there indicated. Hitherto these adsorption relations have been studied by adding dry paper to the staining fluids—Bayliss (1906). This latter method does not give an adsorption comparable with the adsorption of toxin by antitoxin, for the latter is already in the moist condition. If dry paper be added a capillary convection of fluid takes place, likewise an imbibition of the solvent and rapid precipitation of the congo red in the paper. It is important that the nature of the fluid moistening the paper be as nearly the same as that containing the dye. Thus when moistened with fluids containing more or less alcohol than that present in the congo red solution (10 %) convection currents are produced which lead to quite other general laws of staining. That the influence of capillary phenomena should be eliminated in the study of adsorption is well shown by experiments with methylene blue = eosin in methyl alcohol and water, or neutral red = "Licht" green in methyl alcohol and water. A strip of dry filter paper shows differentiation of solvent and the individual

stains; e.g. "Licht" green rises in the wetted paper to a much greater height than the neutral red. Paper moistened with the solvent is more uniformly stained and any difference between the rates of adsorption of the two stains is due to their different rates of diffusion.

The filter paper being regarded as the analogue of the antitoxin and the dye congo red as corresponding to the toxin the Danysz Effect was determined for the staining of the paper by experiments analogous to those of Madsen and Walbum on tetanolysin and its antilysin. The "Effect" for the staining of paper by congo red is expressible by formulae of the same form as those used by Arrhenius for the Danysz Effect in the toxin-antitoxin reaction. It was however found to diminish with diminishing ash content in the filter paper stained, and also with diminishing salt content in the solvent fluid.

The Danysz Effect is explicable from the point of view of adsorption as due to a condensation of the systems consequent on the fixation of the dye in staining paper or of the toxin-antitoxin system after union has taken place and bears a relation to the second phase of agglutination and of precipitation. The first phase of staining is the adsorption of dye, the second a contraction of the stained material which becomes much less fragile, i.e. less easily separated into parts—as can be easily demonstrated. The first phases of the toxin-antitoxin reactions and of agglutination are absorptions, the second phase of the toxin-antitoxin reaction a withdrawal from the medium by contraction of particles by agglutination or coalescence.

The Danysz Effect is then viewed as a physical condensation of the reacting system, having an intimate connection with other phenomena in Immunity.

#### REFERENCES.

- ARRHENIUS (1907). *Immunochemistry*, The Macmillan Co., New York.
- (1908). *Journ. of Hygiene*, Vol. viii. p. 1.
- BAYLISS (1906). *Bio-Chemical Journal*, Vol. i. p. 175.
- BILTZ (1904). *Göttinger Nachrichten*, Math.-phys. Kl. No. 1.  
— *Zeitschr. f. physical. Chemie*, Vol. xlvi. p. 615.
- BORDET (1903). *Ann. de l'Inst. Pasteur*, Vol. xvii. p. 161.
- CRAW (1904). *Lancet*, p. 434.  
— (1905, i). *Journ. of Hygiene*, Vol. v. p. 115.  
— (1905, iii). *Proc. Roy. Soc. B*. Vol. lxxvi. p. 188.  
— *Zeitschr. f. physical. Chemie*, Vol. lii. p. 569.  
— (1907). *Journ. of Hygiene*, Vol. vii. p. 501.

- DANYSZ (1902). *Ann. de l'Inst. Pasteur*, Vol. xvi. p. 331.
- v. DUNGERN (1904). *Deutsche med. Wochenschr.* Vol. xxx. pp. 275, 310.
- EHRLICH (1898). *Deutsche med. Wochenschr.* Vol. xxiv. p. 597.
- (1903). *Berlin. klin. Wochenschr.* Vol. xl. pp. 793, 825, 848.
- HOITSEMA (1895). *Zeitschr. f. physical. Chemie*, Vol. xvii. p. 1.
- LAUDSTEINER (1903). *München. med. Wochenschr.* Vol. l. p. 764.
- MADSEN and ARRHENIUS (1906). *Meddelh. från Vet.-Akads. Nobelinstitut*, Vol. i. No. 3.
- *Communic. de l'institut sérothérapique de l'état Danois*, Extraits, Vol. i.
- (1907). *Centralbl. f. Bakteriol.* Ref. Vol. xxxix. p. 189.
- NERNST (1904). *Zeitschr. f. Electrochemie*, Vol. x. p. 377.
- RAMSEY, MOND and SHIELDS (1895). *Phil. Trans. Roy. Soc.* Vol. clxxxvi. p. 657
- (1897). *Ibid.* Vol. clxl. A p. 129.
- (1898). *Ibid.* Vol. clxli. A p. 105.
- SACHS (1904). *Berlin. klin. Wochenschr.* No. 16.
- *Centralbl. f. Bakteriol.* Vol. xxxvii. No. 2.
- TRAVERS (1906). *Proc. Roy. Soc. A.* Vol. lxxviii. p. 9.

## PLAQUE IN FURTHER INDIA.

BY JOHN D. GIMLETTÉ, M.R.C.S., L.R.C.P. (LOND.),  
*Medical Officer to the Duff Development Company, Ltd., Kelantan.*

FURTHER India has been affected by plague to a small extent in comparison to the Indian Empire. Some considerable interest therefore attaches to the comparatively unimportant local outbreaks which occur, from time to time, in the Malay Peninsula. It is generally very difficult to explain the reason for their limitation and to determine, with any degree of accuracy, the source of the infection. Kelantan, a small but populous and fairly healthy Native Malay State, situated in geographical position between  $4^{\circ} 45'$  and  $6^{\circ} 25'$  lat. N., and  $101^{\circ} 30'$  and  $102^{\circ} 40'$  long. E., has only been exploited by European enterprise during the last seven or eight years. The State seems hitherto to have escaped from plague. The Kelantan Malays in fact, so late as 1905, looked upon plague as a new disease and, for want of a better name, then referred to it as the "penyakit leher" or "neck sickness." Numerous deaths from this so-called "neck sickness" were reported by the natives of the interior to have occurred at some remote villages in the month of May 1905. The illness appeared from the clinical descriptions given by the Malays at the time to have been bubonic plague. It was not thought necessary however by the Kelantan Government to take any special precautions to prevent its spread, and the disease disappeared of its own accord in a few months' time.

Late in 1907, plague broke out among a gang of imported Javanese coolies who had been working throughout the year on a part of the Duff Development Concession, which is situated in the interior of Kelantan. It was at once recognised by the local Malays as the new disease, or "penyakit leher," of 1905.

In this instance, prompt preventive measures were taken by the Duff Development Company and no wide-spread epidemic occurred,

but it is not easy to say whether the limitation was really due to the precautions taken, or to the climatic conditions of the Malay Peninsula, which seem to prevent the spread of plague in an indirect way, or to the keeping of cats—a co-operative measure which as Lieut.-Colonel Buchanan (1908) has lately pointed out—seems to have some indirect controlling influence over plague in India.

The plague in Kelantan occurred on a part of the Duff Concession, which is being opened up by the planting of a rubber estate, situated about one hundred and fifty feet above sea level and sixty miles inland, located on the left bank of the main river and some fifty miles from the capital of the State. Among many commercial possibilities in Kelantan the planting of rubber is attended with success because, speaking generally, the earth is fairly loose, and although poor in chemical composition, like the generality of soils elsewhere in Malaya, is well adapted by favourable physical and meteorological conditions to the successful growth of the trees.

In 1907, the total rainfall for the interior was 120·54 inches, with 176 rainy days; the mean average maximum temperature in the shade for the year was 85·7° F., and the average mean minimum temperature 70·9° F.; shade maximum temperature 90·5° F., and minimum 67·0° F. The climate is therefore naturally suitable for planting in being uniformly hot as well as constantly moist, a condition which appears to be unfavourable to the propagation of the fleas which are so commonly associated with man and domestic animals in the hotter, drier and more sandy parts of the tropics.

The flea (*Pulex irritans*) as a parasite of man and as distinct from the bug ("pijat," in Malay), is unknown to Malays in this part of Further India. There is no distinctive name for it, but the dog flea is well known as "kutu anjing" (lit. "dog louse"), probably because it is the most common species; the lighter coloured cat flea is not specially identified by Malays, neither is the smaller black goat flea commonly found on kids. A rat flea again is not specially recognised, although rats as well as mice are generally to be found in the bamboo huts of any Malay village community. A certain amount of paddy, or rice in the husk, is always stored either within or underneath the houses, which are almost invariably raised some feet from the ground.

The keeping of cats is a national Malay convention which is common to other Mohammedan people, but in Malaya the influence of folk-lore has an additional significance. The ancient superstitious faith of the Malays before their conversion to Mohammedanism seems to survive

in the everyday treatment of their cats and to afford them some protection.

Among many curious ancient Malay beliefs concerning mammals, is one that an evil genius pertains to both the rat and the cat. Clothes, for instance, however costly, that have been nibbled by rats must not be worn again. A cat is always driven out of the house in the event of a death; it is most unlucky if perchance a cat should brush against a corpse, the dead may stir. It is even more unlucky to kill a cat outright, and consequently at least one or two ill-favoured cats are to be found in almost every Malay dwelling-place.

The fact that they kill rats is the reason which is invariably given for their tolerance.

In the Malay State of Kedah there is even a special exclamation, "puspas," for driving away the cat from the primitive household dresser, but should it become too tiresome any Malay cat is banished from the village by common consent. As a last resource, it is tied, in Kelantan, by the neck to a stake, fixed on an improvised raft, and floated down stream. The unfortunate village cat again is not unfrequently drenched with water in the hope, by this means, of averting a threatened period of drought. There happened to be no cats on the Company's rubber estate: a fact which is interesting as giving negative support to Lieut.-Colonel Buchanan's hypothesis.

In 1907, for the development of the plantation, about 140 Javanese, 120 indentured Chinese coolies or "sinkhehs," and a variable number of local Malays were employed in clearing, burning and draining the jungle.

The conditions of labour necessarily involved in the development of a new territory were rough and ready, and the health of the imported coolies had in consequence not been good.

Early in November, three deaths occurred suddenly among the Javanese, followed by three others within twenty-four hours. Damp and comparatively cold wet weather, which is usual in the North East Monsoon period, had now set in, and these sudden deaths marked the commencement of a small outbreak of plague which eventually caused 13 deaths out of 31 cases as shown in the accompanying table.

The epidemic began at the time of the Malay "hari raya," an annual native festival which follows the "bulan puasa," or regular Moham-madan fasting month. The occurrence was at a particularly wet season of the year when a migration of rats to the coolie lines, owing to a scarcity of food in the field, might have been thought of, but the early

diagnosis of plague was more than usually difficult because the early symptoms happened to be masked by those of acquired alcoholism.

The type at first was pneumonic and very fatal, followed by an evident bubonic but very mild form of plague and finally resolving into pestis minor.

The Javanese on the estate held the national holiday on the 8th, 9th and 10th days of November, and many of them, not being very strict Mohammedans, indulged in some cheap brandy in order to celebrate their merrymaking. Two boys, Kassim and Kasti, were admitted to hospital on the 10th of the month for pneumonia and pleurisy, but no special importance was attached to their cases; both however died of plague, one on the 13th and the other on the 14th of November. On the 11th, Sakimin, a male dancer, died suddenly at the estate, and his death was supposed by the Superintendent to be due to alcoholism with heart failure from over-exertion in dancing and exposure to the rain. Another male dancer, Mortostiko, died suddenly on the following day. At autopsy alcohol was found in the stomach and there was consolidation of both lungs, but no distinctive buboes being noticed, the real cause was again mistaken and the cause of death returned as pneumonia. On the evening of the same day a third dancer, Matjas, a tailor by trade, died suddenly at the plantation: the autopsy revealed recent pleurisy and dilatation of the heart with acute congestion of the lungs and kidneys. A number of enlarged, deep reddish, lymphatic glands were found in the retro-peritoneal fossae and the diagnosis of plague was made, but there were no characteristic buboes. There was an open wound on the left leg. Other deaths followed in quick succession, all with pulmonary symptoms; Kasti on the 14th, Wagio on the 15th with broncho-pneumonia and diarrhoea, Sutaruno on the 17th—the most typical and virulent example which had so far occurred—Nagadikon on the 20th with leading symptom of pleurisy, Tipostiko on the 21st with pneumonia, Kamso on the 22nd with pleurisy and pneumonia, and Matsariwi on the 23rd with pneumonia. Matsariwi was the last case of the kind, and although no alcohol had been taken, his primary symptoms much resembled those of drunkenness. The autopsy of this Javanese revealed the presence of a horseshoe example of "solitary kidney" which was bound by adhesions to the spinal column. The bad effect of alcohol in plague, other than in medicinal doses, appears to have been only relative in this small epidemic. Out of seven dancers (Kasanpaiviro, Katimin,

Mongin, Mortostiko, Matjas, Sakimin, and Samirah), four had regaled themselves with brandy, and of these only two died, one of them (Katimin), in whose sputa *B. pestis* was found, recovered and the other escaped infection. Of the remaining three, who all drank syrup and water, only Matjas the tailor died suddenly and the other two remained in good health. One of the two that escaped, Samirah, is a woman and it is remarkable that the epidemic, with the one exception of Parsem, a girl coolie and a prostitute, was confined to the male coolies. The children, about fifteen in number, all escaped except one; they lived with the women at the further end of the coolie lines. One little boy, Kader, having no mother, lived with his father in the Javanese male quarters; he fell ill but recovered in hospital from an attack of broncho-pneumonia complicated with large cervical buboes. It was not difficult to demonstrate the presence of *B. pestis* in the sputa of these cases, but no very profuse sanguous expectoration was observed in any of them.

Although plague never occurs in equatorial regions in a bad epidemic form, the gravity of individual cases is maintained. This was exemplified in the case of Sutaruno, a strong man who was brought from the estate as suffering from mumps. *B. pestis* was found in the secretion of the parotid buboes, and hyperpyrexia very rapidly ran into delirium followed by coma and death within twenty-four hours of his admission to hospital. Shortly before his death large numbers of small, superficial, subcutaneous haemorrhages occurred, depicting, in livid colours, the malignant nature of the case. Four days afterwards, Hamat bin Janka, an in-patient under treatment for rheumatism, who had volunteered to nurse this case, developed cervical buboes followed by inguinal buboes but ended in recovery. In the first week there were eight Javanese deaths out of fourteen cases; and of these fourteen cases all except five were characterised by pulmonary symptoms and nearly all were under the age of thirty. A typical example of the sudden collapse which may occur in plague was afforded by Kamso, a young Javanese who left the estate in a quarantine boat for admission to hospital, apparently in no very precarious condition. He died suddenly on landing through being allowed to walk up the river bank which is rather steep. No evident bubonic swellings could be seen or felt after death, but dissection disclosed a mass of deep glands in the right femoral region; both lungs bound down by thick yellow adhesions; the pericardium very much thickened and containing an excess of fluid, the heart dilated; both lungs oedematous and hyperaemic with

haemorrhagic infarcts in the lower lobes, the liver enlarged and both kidneys congested.

The earliest cases of a simple bubonic nature were noticed among the Javanese between the 15th of November and the 8th of December; there were eight altogether and with the exception of one (Kromorjo), they all recovered; four had inguinal buboes, three femoral and one an axillary bubo.

The virulence of the epidemic appears to have been exhausted by the end of November, shortly after recommendations for the prevention of the spread of plague had been very strictly put into force. Up till now the Chinese "sinkhehs," who lived in separate quarters but quite close to the Javanese males, had all escaped; they as well as the Malays employed on the estate were all males, but the Malays dwelt at the further end of the lines near the female quarters about two hundred yards from the other coolies. There was consequently very little intercourse between the Malays and the male Javanese.

Pestis minor was diagnosed mostly among the Chinese "sinkhehs" who began to sicken with it in December, and of those coming within the incubation period (six to seven days) and isolated as "suspects," four developed buboes which could not be accounted for otherwise; these comprised two inguinal, one femoral and one axillary bubo. About the same time, some Chinese employed at the headquarters of the Company, nearly three miles from the estate, fell ill and four other cases were found, three with femoral and one with cervical buboes, but none of these men suffered much inconvenience; all recovered and several on admission might, by casual clinical examination, have been easily mistaken as suffering from malaria. Four of the total number were treated for double femoral buboes.

The actual channel through which the infection was imported to the estate was not determined. Popular suspicion rested upon some clothes brought in by Matjas the tailor, who went to Kota Bharu the capital, fifty miles away, to purchase goods for the coming "hari raya" festival. Kota Bharu was known to be free from plague by the Siamese medical officer and the resident English officers representing the Government of Kelantan, and he returned from there on October 25th, sixteen days before the first case. His purchases consisted of a few pounds of some pleasant tasting seeds (basil), some syrup, sugar and a variety of clothes among which were three pairs of elastic braces. The clothing was not however unpacked until the first week in November, it was then put up for sale at the estate, and Samirah, the dancer, bought and wore some of it with no ill effects; Parsem wore some and

was very ill, but the latter being a prostitute it is probable that she may have acquired infection in some other way. The braces, said to have been imported from Bombay *via* Singapore, were bought by two of the men who died suddenly and the odd pair was given to a little boy (Jonet), a nephew of the deceased tailor.

All the clothing was sold, along with the other purchases, at a small native shop kept on the estate, at that time, by the Javanese headman. The shop was in a very insanitary condition, more especially in regard to ventilation, but although rice and other native food stuffs, such as cakes, sweetmeats, biscuits and the like, were stored, it never appears to have been over-run with rats. No mice were seen. A possible hiding place for fleas existed in some bullock hides which were stored among the rafters of the house.

In 1905, most of the deaths occurred inland on the opposite bank of the river, but a few Malays died from "penyakit leher" in a village near the site afterwards chosen for the dwelling houses on the plantation. This suggests that the former epidemic may have subsided owing to the heat of the South West Monsoon period of 1905 and, the infection remaining latent in 1906, may have revived during the coolest part of the year 1907.

Presupposing that a number of rats existed in the field, the hypothesis is not so improbable as it may seem at first sight. The infection, for instance, may have remained latent in the form of chronic rat plague. In 1907, the last burning of felled timber and brushwood on the plantation took place in the forenoon, late in October. It would certainly have driven any field rats from their burrows and may have caused them to seek shelter in the coolie lines which happened to be near at hand. Fleas imported by traders from without and introduced among the clothing brought in to the estate by Matjas the tailor may then have been infected.

The Company's hospital being situated up stream, a system of inspection in conjunction with a quarantine boat service was at once established; the patients were isolated and a crusade commenced against the rats found in the houses on the estate. This was done by means of traps and by killing them with sticks by hand. It disclosed some features of interest, as there were apparently very few rats, either in the shop or in the adjacent Javanese male quarters. No attempt was made to destroy rats in the field. The wet weather was unfavourable for the practice of suffocating them in their tunnels by means of carbon bisulphide. This method, which is used for the extermination of rats in the rice fields of the Federated Malay States, was recently introduced by the Director of Agriculture and would, in other circum-

stances, be also of material benefit in checking the spread of plague. Out of a total of about fifty rats eventually killed on the estate, the majority came from the female quarters when the epidemic was practically over. They were nearly all young rats about the age of commencing sexual maturity and all of them were small black rats (*? Mus rattus*). A much larger brown rat, which is found in the native town of Kota Bharu, was not seen on the estate. It was of interest to find that most of the few rats that were caught in December were young ones because it has been shown in Egypt that the maximum pregnancy of the rat, in that country, corresponded with the maximum of plague and the literature of plague abounds with examples of epidemics in which a scarcity or disappearance of rats has been noted. No naked eye appearances of disease were recognised in four adult rats that were dissected or in five others that were examined; *Loemopsylla cheopis*, the flea of the black rat, escaped recognition although a sharp look-out was kept for its capture. For the destruction of these insects all the coolie lines were disinfected bi-weekly with a solution of Jeyes' fluid, about 1 in 40 in strength.

The hides were disposed of; all clothing which in any way appeared likely to convey infection was burnt, the shop pulled down and a number of minor precautions of local importance insisted upon. Quarantine regulations were dispensed with one month after the diagnosis of the last case, but still a good deal of anxiety was felt for the Malays on the estate who suffered from an ill-defined form of fever in the middle of December. They disbanded, returning to their homes to be nursed by their women folk as is their custom. Kelantan peasants are very conservative in their customs and in their belief of ancient superstitions, such, for example, as breathing or blowing over a cadaver in order to drive out a supposed evil familiar spirit; this and similar procedures, employed under exceptional circumstances, would render the scientific practice of plague prevention very difficult in Further India in the event of a serious epidemic of plague occurring among the Malay residents. Out of about forty Kelantan Malays however who might have been exposed to infection at the estate, happily only one death from "penyakit leher" was announced from the neighbouring villages and there has been no recurrence of the disease.

#### REFERENCES.

- BUCHANAN, A. (24. v. and 24. x. 1908). Cats as Plague preventers. *Brit. Med. Journal*, i. p. 1285, ii. p. 1231.
- GALLAGHER, W. J. (III. and XI. 1908). Extermination of Rats in Paddy Fields. *Agricultural Bulletin of the Straits and Federated Malay States*. Nos. 3 and 11.

## Plague in Further India

A return showing the number of cases of Plague in Kelantan—1907.

No.	Name	Sex	Nationality	Occupation	Date of attack	Result	Clinical Remarks
1	Kassim	M	Javanese	Estate Coolie	10. xi. 07	Died 13. xi. 07	Leading symptom, pneumonia.
2	Kasti	M	"	"	10. xi. 07	Died 14. xi. 07	Leading symptom, pneumo-pneumonia.
3	Sakimin	M	"	"	11. xi. 07	Died 11. xi. 07	Died suddenly, leading symptom, alcoholism.
4	Katimin	M	"	Mandore	12. xi. 07	Recovered	Leading symptom, pneumonia; <i>B. pestis</i> in spina.
5	Mortostiko	M	"	Estate Coolie	12. xi. 07	Died 12. xi. 07	Leading symptom, alcoholism, p.m. exam.
6	Matias	M	"	Tailor	13. xi. 07	Died 14. xi. 07	Died suddenly at the Estate, p.m. exam.
7	Wagio	M	"	Estate Coolie	14. xi. 07	Died 15. xi. 07	Leading symptom, broncho-pneumonia.
8	Kader	M	"	Child	15. xi. 07	Recovered	Pneumonia and cervical buboes.
9	Wongsaeksomo	M	"	Estate Coolie	15. xi. 07	"	Indolent femoral buboes.
10	Sutiaruno	M	"	"	16. xi. 07	Died 17. xi. 07	Septicemic, large cervical buboes, p.m. exam.
11	Matsarawi	M	"	"	17. xi. 07	Died 23. xi. 07	Pneumonia with deep glands, p.m. exam.
12	Kasanpanviro	M	"	"	17. xi. 07	Recovered	Indolent inguinal buboes.
13	Madicom	M	"	"	17. xi. 07	Died 20. xi. 07	Acute pleurisy with deep glands, p.m. exam.
14	Kronorio	M	"	"	17. xi. 07	Died 3. x. 08	Indolent inguinal buboes, asthenia.
15	Tipostiko	M	"	"	19. xi. 07	Died 21. xi. 07	Leading symptom, pneumonia.
16	Matsojoh	M	"	"	19. xi. 07	Recovered	Bubo, right axilla, <i>B. pestis</i> found.
17	Mamat bin Janka	M	"	"	20. xi. 07	"	Contracted in hospital from case No. 10; cervical and femoral buboes.
18	Kamso	M	"	"	22. xi. 07	Died 22. xi. 07	Died suddenly; p.m. exam., pleuro-pneumonia.
19	Parseem	F	"	"	22. xi. 07	Recovered	Indolent cervical and inguinal buboes.
20	Hamat Estart	M	"	"	23. xi. 07	"	Indolent inguinal buboes.
21	Slamat	M	"	"	26. xi. 07	"	Indolent inguinal buboes.
22	Kasannmursadi	M	"	"	27. xi. 07	"	Indolent femoral buboes.
23	Taiio	M	"	Sinkkeh	4. xii. 07	"	Femoral buboes; <i>B. pestis</i> found.
24	Liang Ho	M	Chinese	"	8. xii. 07	"	Inguinal buboes; subsequently died of Beri beri.
25	Wong Kong	M	"	Prisoner	8. xii. 07	"	Indolent femoral buboes.
26	Lee Ah Choi	M	"	Barber	10. xii. 07	"	Indolent femoral buboes.
27	Tan Kit	M	"	Sinkkeh	15. xii. 07	"	Bubo, left axilla; subsequently died of Beri beri.
28	Ang Niu Seng	M	"	"	15. xii. 07	"	Indolent femoral bubo; died of Beri beri.
29	Hai Thiam	M	"	Station Coolie	15. xii. 07	"	Bubo, left groin.
30	Chan Yow	M	"	Sinkkeh	17. xii. 07	"	Indolent inguinal buboes.
31	Chin Heng	M	"	Station Coolie	23. xii. 07	"	Cervical buboes.

## AN ANTI-SERUM FOR SCORPION VENOM.

By CHARLES TODD, M.D. (CAMB.), D.P.H.,  
*Bacteriologist, Public Health Department, Egyptian Government.*

MANY deaths occur every year in Egypt as the result of scorpion sting. These deaths are practically confined to Cairo and Upper Egypt, and are almost entirely among children. Unfortunately, owing to local conditions, it is almost impossible to obtain really accurate figures for this mortality, but some idea may be formed from the statistics given below of some of the larger towns.

The mortality in the country is probably considerably greater than in the towns, which have many European houses where the scorpion naturally does not find such favourable conditions for its existence as in the small mud huts of the villages.

The following are the statistics for some of the larger Egyptian towns, for a period of seven years, viz.: 1901—7<sup>1</sup>.

Name of town	Population	Deaths from scorpion sting 1901—7	Per 1000 per annum
Cairo	600,000	153	0·036
Fayoum	35,000	29	0·120
Beni-Souef	19,000	18	0·180
Minia	25,000	15	0·080
Assiout	47,000	87	0·260
Sohag	15,000	33	0·320
Keneh	33,000	46	0·200
Assouan	18,000	63	0·640

Some of these figures are very high, notably in the case of Assouan, with 0·64 deaths per 1000 of the population, which represents 1·6 % of the total death rate. The deaths occur almost entirely among children under 12 years of age though adults occasionally die.

<sup>1</sup> Births and Deaths in the principal towns of Egypt, 1901—7, Statistics Department of Public Health.

Apart from the mortality which it causes, the scorpion is a very serious pest, for even when the sting is not fatal it gives rise to very great pain, and often to severe collapse.

In the summer of 1906, Sir Horace Pinching, then Director General of the Egyptian Public Health Department, suggested to the writer the investigation of the venom of the scorpion, with a view to the possibility of preparing an anti-serum for use in Egypt.

Arrangements were made for the collection of scorpions in Upper Egypt, and as soon as sufficient of these were available, the immunisation of animals was commenced.

#### *Historical.*

Many scattered references to the effects of scorpion stings are found in the early literature of medicine, but the first systematic investigation of the venom appears to have been undertaken by Valentin (1876), Joyeux Laffuie (1882), and Paul Bert (1885). Later, the venom was more fully investigated by Wilson (1904), whose monograph on the subject includes a very complete account, not only of the character of the venom, but also of its effects on isolated nerve and muscle.

#### *Preparation of scorpion toxin.*

According to Wilson (1904, p. 11) of the numerous species of scorpion found in Egypt, only three are at all common, viz.:—

"(1) *Buthus quinque striatus*. Common throughout the country, more especially in Upper Egypt.

(2) *Prionurus citrinus*. Found in the desert near Cairo, and in the neighbourhood of Alexandria.

(3) *Buthus maurus*. A black scorpion which seems to be particularly common at Mariout to the west of Alexandria, but which is also found at Sakkara, south-west of Cairo.

*Buthus quinque striatus* is undoubtedly the common variety, and is generally thought to be the most dangerous; it is frequently found in houses and is the species in all probability giving rise to the numerous cases of scorpion sting said to be not uncommonly fatal in Upper Egypt.

The adult size of the three species mentioned is about the same, the maximum length in each case being about 10 cm.

*Buthus quinque striatus* is of a sandy yellow colour tending to

brown, the segments of the tail being roughly cylindrical in section, the telson or sting being relatively large.

*Prionurus citrinus* is of a greenish yellow colour, the tail segments being broad and carinated, and the sting well developed.

*Buthus maurus* is almost black on the upper surface, brown on the under surface of the abdomen, the comb-like sense organs attached to the abdomen just behind the posterior limbs being of lemon-yellow colour, and the tail segments broad with a deep median groove the lateral ridges of which are distinctly serrated, and the sting relatively small."

In collecting scorpion venom on a large scale it was impossible to make any determination of species, as the scorpions were obtained from various towns of Upper Egypt by means of a small reward given to people bringing them in to the government medical officers.

The latter, with a pair of scissors, snipped off the sting with the last joint of the tail. The stings and venom glands so obtained, after being dried for a day or so in the sun, were posted to Cairo, and there placed in dessicators over calcium-chloride where they were kept until required for use.

When a solution of the venom was required, the dried stings and venom glands were ground to a fine powder in a Turkish coffee mill, and added to a 0·8 % saline solution, in the proportion of one sting to 1 c.c. of saline solution.

The resulting suspension was agitated by means of a mechanical stirrer for one or two hours, and then centrifuged. The supernatant opalescent fluid was at first filtered through a Berkefeld or Chamberland candle, but later it was found more convenient to clarify it by the addition of a little aluminium sulphate and lime water in order to get rid of any spores that might not have been removed by the centrifuging. It was then placed in stoppered bottles with a little chloroform, and kept in the ice-safe.

In grinding large numbers of the glands, it is advisable to have a closed mill, as the resulting powder even in the smallest quantity is exceedingly irritating to the nasal mucous membrane, giving rise to violent coryza.

Prepared by the above method, the venom was found to be very stable, and the method is more practical than extraction by glycerine, particularly when the venom is to be employed for immunisation of animals, as before use the chloroform is easily removed by allowing filtered air to bubble through the solution.

As a certain amount of decomposition had often occurred in the tails before they became dry, the solution obtained was far from being a pure solution of venom; but the various methods of purification which were tried (e.g. precipitation by alcohol, ammonium sulphate, etc.) were found either to be too complicated for practical use, or to result in too great a loss of the venom, and as any products of decomposition remaining in the solution were not present in sufficient quantities to cause inconvenience the simpler method was adhered to.

#### *General characteristics of the venom.*

The venom is soluble to some extent in distilled water, i.e. it may be extracted from the glands by rubbing them with water, but as was pointed out by Wilson (1904, p. 13) it is much more completely extracted by normal saline. It is also soluble in glycerine.

Like the bacterial toxins, it is precipitated by saturating its solution with ammonium sulphate, or by pouring the solution into excess of alcohol.

The venom passes through a Chamberland filter without great loss.

In one experiment, the venom glands from 400 scorpions were powdered and shaken with 400 c.c. of 0·8 % saline solution, and the suspension allowed to stand over night in the ice-safe. Next morning the supernatant opaque fluid was tested on young pigeons and 0·3 c.c. was found to kill a pigeon of 250 grammes in two hours.

The fluid was then divided into portions which were filtered through Chamberland and Berkefeld candles respectively.

The Chamberland filtrate killed a pigeon of 250 grammes in a dose of 0·5 c.c. in 80 minutes, while of the Berkefeld filtrate almost 1 c.c. was required. This experiment was repeated with similar results. It is interesting to note that the toxin appears to pass through the close grained porcelain Chamberland filter more easily than the comparatively porous Berkefeld, but it is possible that this is accounted for by some absorption in the case of the Kiesselguhr filter.

Compared with the bacterial toxins, scorpion venom is very stable, and can be kept in solution in the ice-safe for many months with only a slight loss of toxicity.

It is unaffected by drying, and as was shown by Wilson, is very resistent to putrefaction, and is not affected by heating to 100° C. for short periods, but it is destroyed if the heating is continued from 12 to 13 minutes.

One cubic centimetre of scorpion extract, which killed a guinea-pig of 400 grammes in 20 minutes, when given subcutaneously after being heated for an hour at 90° C. killed an animal of the same weight in 1½ hours.

As has already been mentioned, chloroform and glycerine appear to have no action on the venom, but it is very susceptible to certain other chemicals: e.g. ammonia and iodine.

Very small quantities of iodine render the venom quite inert, as is shown in the following experiment, where 0·5 c.c. of scorpion extract was mixed with varying quantities of ordinary Gram's solution, and after standing for one hour, injected subcutaneously into guinea-pigs of from 340 to 360 grammes weight:—

Guinea-pig	Mixture used	Effect
No. 1	0·5 c.c. scorpion extract with 0·2 c.c. Gram's solution  (Mixture showed faint iodine tint.)	Lived. No symptoms.
No. 2	0·5 c.c. scorpion extract with 0·1 c.c. Gram's solution  (Mixture colourless.)	Lived. No symptoms.
No. 3	0·5 c.c. scorpion extract with 0·075 c.c. Gram's solution  (Mixture colourless.)	Died in 60 minutes.
No. 4	0·5 c.c. scorpion extract with 0·05 c.c. Gram's solution  (Mixture colourless.)	Died in 45 minutes.
No. 5	0·5 c.c. scorpion extract with 0·02 c.c. Gram's solution  (Mixture colourless.)	Died in 30 minutes.

As the venom of one scorpion is represented by 1 c.c. of the extract, this quantity is completely neutralised by 0·2 c.c. of Gram's solution, corresponding to two thirds of a milligramme of pure iodine.

#### *Susceptibility of various animals to the toxin.*

Most of the vertebrates which have been tested, with certain exceptions, are susceptible to scorpion venom, though the degree of susceptibility shows considerable variation in the different species.

The *horse* is highly susceptible: in one case 1 c.c. of a toxic extract, corresponding to the venom of one scorpion caused very severe illness, with marked salivation. The *goat* is considerably less susceptible, the subcutaneous injection of a dose of 5 c.c. of a similar extract into a young

animal only causing it pain, profuse salivation and straining: the next morning the symptoms had completely disappeared. The *dog* is susceptible, but the susceptibility appears to vary in different individuals. The *common fox* was found by Wilson to be susceptible. The *mongoose* is very resistant, but not completely immune. The *guinea-pig* is very highly sensitive to the venom, young animals of 300 grammes being killed by 0·05 c.c. of the extract (i.e. 1/20 of the total poison of a scorpion). The *rabbit* is more resistant than the guinea-pig. 1 c.c. of the extract being required subcutaneously to kill an animal of 1000 grammes. The *white rat* and *white mouse* are highly susceptible. *Mus alexandrinus* and *Mus musculus* are both susceptible. The *pigeon* is very susceptible: 0·17 c.c. of the toxic extract being sufficient to kill a bird of 240 grammes. A *tortoise* (*Testudo graeca*) weighing 1275 grammes was injected subcutaneously with 2 c.c. of somewhat weak extract and found dead next morning. A large *lizard* (*Uromastyx spinifex*) showed very marked symptoms after an injection of 3 c.c. (Wilson). *Frogs* and *toads* were found by Wilson to show marked symptoms after a large dose of the poison.

#### *Animals immune to the poison.*

A very interesting observation was made by Wilson who showed that certain desert animals, which live under conditions which must constantly bring them into contact with scorpions, possess a high degree of immunity to scorpion venom.

This he showed to be the case with the following:

- Desert rat. (*Gerbilus pyramidum.*)
- Jerboa. (*Jaculus jaculus.*)
- Fennix Fox. (*Vulpes zerda.*)
- Zarilla. (*Ictonix lybica.*)
- Hedgehog. (*Erinaceus auritus.*)
- Varanus cinereus.*

In this connection it is interesting to note that the *Acomys cahirinus* (Prickly mouse) which is very common in native houses, shows a very marked immunity to the venom, a dozen of 0·5 c.c. of a toxic extract (corresponding to half the total venom of a scorpion) gives rise to no symptoms, whilst the ordinary mouse (*Mus musculus*) and rat (*Mus alexandrinus*) are very susceptible to the poison.

A non-poisonous snake (*Zamenis*) which received 2 c.c. of scorpion extract showed no symptoms of any kind and remained well.

*Action of the toxin on susceptible animals.*

This will not be gone into here, as the physiological action of the venom has been investigated by Wilson, who, in his monograph, gives very complete details not only of the symptoms in animals, but also of the action of the toxin on isolated muscles and nerves, as well as the post-mortem appearances.

He summarises the symptoms as follows, in order of their appearance.

- (a) Local irritation.
- (b) Muscular twitching, chiefly confined to the head and neck.
- (c) Jumping movements.
- (d) Lachrymation.
- (e) Milky orbital secretion. Nasal secretion, and salivation.
- (f) Prolonged muscular spasms; most marked in the hind limbs, but affecting the muscular system generally.
- (g) Erection of hair; especially on the fore part of the body and face, to which it gives a peculiar swollen appearance over the jaws.
- (h) Passage of liquid faeces (often absent).
- (i) Erection of penis, and discharge of semen.
- (j) Apparent paralysis; the animal lying on the side, abdominal muscles usually tense; and breathing shallow; expiration prolonged.
- (k) Symptoms of asphyxia; blueness of mucous membranes. Convulsions; intermittent gasping respiration.
- (l) Cessation of respiratory movements. Gradual slowing and stoppage of heart-beat.

Talaat (1904) concludes that the toxin acts on the nerve centres, especially the medulla and spinal cord, and in support of this view he gives the following experiment:—

“A guinea-pig of 750 grammes was stung by a scorpion and died after 70 minutes, after showing all the symptoms of acute poisoning. The medulla and spinal cord were removed and allowed to macerate for 7 days in 25 c.c. of glycerine. After this time 1 c.c. of the glycerine extract injected intraperitoneally into a guinea-pig whose weight is not stated was found to cause death with typical symptoms in 60 minutes.”

Now in the above experiment the animal that was stung by the scorpion did not die until 70 minutes after the sting, showing that it

could not have received more than two or three times the minimal lethal dose of the venom: the glycerine extract of the medulla and the spinal cord however, according to the test on the second guinea-pig, contained at least 25 minimal lethal doses, which would mean that an actual increase of the venom had taken place in the body.

There does not appear to be any evidence of the fixation of the scorpion venom by the central nervous system as was shown by Wassermann to occur in the case of tetanus toxin. This is seen in the following experiment:—

The brain of a healthy guinea-pig was rubbed down to an emulsion in a mortar with as small a volume of normal saline as possible, and the following mixture made:—

2 c.c. scorpion extract,  
5 c.c. brain emulsion, and  
3 c.c. normal saline.

The mixture was kept at 37° C. for an hour, at the end of which time it was centrifuged, and the supernatant fluid decanted. The deposit of brain substance was washed once with fresh saline, and again centrifuged, and the deposit made up to the same volume as the original supernatant liquid. The supernatant fluid from the first centrifugalisation and the washed deposit of brain substance were then tested on guinea-pigs.

#### *Supernatant fluid.*

- |   |                      |
|---|----------------------|
| No. 1. Guinea-pig : 2 c.c. intraperitoneally. | Died in 90 mins.     |
| No. 2.        "        4 c.c.        "        | "        45        " |

#### *Washed deposit.*

- |  |                     |
|--|---------------------|
| No. 3. Guinea-pig: 2 c.c. intraperitoneally. | Lived, no symptoms. |
| No. 4.        "        4 c.c.        "       | "        "        " |

A further experiment was made in which a young guinea-pig (350 grammes in weight) was inoculated with 2 c.c. of scorpion extract (T. 19) which represented over 20 M.L.D. Death occurred in 19 minutes. The medulla and spinal cord were at once emulsified in 5 c.c. normal saline, and the whole mixture injected intraperitoneally into another guinea-pig. This animal remained well, and showed no symptoms.

*Action of the venom on the blood.*

Unlike snake venom, scorpion venom appears to have no effect on the coagulability of the blood, nor does it appear to have any haemolytic action on the red blood corpuscles.

The corpuscles of the ox, sheep, guinea-pig, rabbit, and pigeon were tried, but showed no trace of haemolysis. This is in accordance with the observations of Paul Bert, and Wilson.

Flexner and Noguchi (1902) showed that snake venom has no action on certain red blood corpuscles if these are thoroughly freed from serum, and that the haemolysis which takes place, when serum is present, must be due to the complement like action of some substance in the serum. Calmette (1902) showed that this substance, unlike ordinary serum complement, is not destroyed by heating to 62° C., and Kyes was able to identify it as a lecithin. Kyes (1903) then made experiments with scorpion venom, and found that this venom which was only slightly haemolytic for guinea-pigs' corpuscles, and without any action on other corpuscles, on the addition of lecithin became haemolytic for all the corpuscles on which it was tested, and almost equally for all, being about 20 times weaker than cobra venom.

He was also able by the usual method to prepare qualitatively a typical lecithide of the scorpion venom. Kyes does not mention the species of scorpion with which these experiments were made, but states that they were obtained from the Botanical Gardens of Buitensorg. These experiments of Kyes have been repeated by the writer with the mixed venom of the Egyptian scorpions, but the results so far have been quite negative as regards the complement-like action of lecithin, as is shown by the following experiments, made with washed guinea-pigs' corpuscles.

*Action of scorpion venom, with and without lecithin,  
on guinea-pig corpuscles.*

Guinea-pigs' red blood corpuscles three times washed with normal saline solution. Scorpion venom filtered through Berkefeld candle.

Merks' "Ovolecithin" 1 %	Scorpion toxin	Scorpion toxin+lecithin
1 c.c.	No trace of haemolysis	No trace of haemolysis
0·5 c.c.	" "	" "
0·2 c.c.	" "	" "
0·1 c.c.	" "	" "
0·05 c.c.	" "	" "
0·02 c.c.	" "	" "
0·01 c.c.	" "	" "

In this experiment the presence of lecithin appears to have no action in producing haemolysis. The same experiment was repeated with rabbits' red blood corpuscles with similar negative results.

It was then thought that possibly the lecithin was at fault, and the experiments were repeated with *afga lecithin* as recommended by Kyes, but again with negative results.

An attempt was then made to prepare a scorpion lecithide, using the method employed by Kyes. The poison of about 80 scorpions was used. The stings were ground, extracted with saline, the extract filtered through cotton wool, and then evaporated to dryness over sulphuric acid. The dried extract was ground in a mortar with distilled water, and mixed with about half its volume of 15% *afga lecithin* in chloroform, and kept over night in the incubator, but from time to time being shaken up. The next morning this was centrifuged, the clear chloroform layer pipetted off and poured into 6 to 7 times its volume of pure ether. Not the slightest trace of precipitate was formed.

The difference between these results and those of Kyes may be due to the different species of scorpions used, as in his paper Kyes does not state what scorpion he worked with.

The venom does not appear to have any marked action on the coagulability of the blood.

#### *Action on the mucous membranes.*

As has already been stated the powdered glands are intensely irritating to the nasal mucous membrane, so much so, that before a well-closed mill was used for grinding the glands, it was found necessary to apply a layer of cotton wool to the nostrils to act as a filter during the grinding process.

The toxic extract of the glands (1 c.c. representing the venom of one scorpion) seems however to have very little irritant action upon the conjunctiva, as it did not give rise to any obvious redness, when dropped into the conjunctival sac in the rabbit or guinea-pig.

#### *Anti-venom. Historical.*

In most countries where scorpions are common, persons are found who handle these animals quite freely and without fear. It is somewhat difficult to know whether this is due to any actual immunity to the venom, or merely to the knowledge of the creature's habits, and a certain skill in handling.

The above does not refer to the Arabs who exhibit scorpions in the streets of Cairo for the entertainment of tourists, as in this case the scorpions will generally be found to have the points of their stings cut off, so that they are practically harmless.

It seems however that individuals do exist who have a true acquired immunity. Schinz (1891) states that in German S. W. Africa a number of the population render themselves immune to scorpion poison. Wilson (1904, p. 38) referring to this matter says:—

"In India, South Africa, and probably Egypt, a certain number of individuals among natives have methods of rendering themselves immune to the venom. The two men from whom I have obtained scorpions handle them without any fear; one of them informed me that he was unaffected by scorpion stings, as he had accustomed himself to the venom by pricking the skin from time to time with the sting removed from a dead scorpion; he stated that he carried the stings with him when on a journey, since if he failed to use them for any length of time he would lose the protection conferred upon him. I have never seen this man stung by a scorpion; whether the process would be sufficient to confer immunity is difficult to say: I find however that a small guinea-pig may be killed by pricking the skin with a sting recently removed from a scorpion, even if care be taken to avoid expressing any venom from the gland. It is probable therefore that there is always a certain quantity around the point at which the venom ducts open."

From this it may be regarded as probable that pricking the skin with a sting from a dead scorpion would introduce a small amount of venom into the system, and no doubt gradually confer immunity."

The natural immunity of certain cold-blooded animals has already been referred to, and the scorpion is known to be immune to his own venom.

It is a popular belief that when a scorpion finds himself in a position where death appears inevitable, that he commits suicide by stinging himself, and that this can easily be demonstrated by placing a scorpion on any flat surface, and surrounding him with a ring of burning spirit. After running about, and finding no way of escape, he is said to curl his tail over and sting himself in the body.

This question was investigated experimentally by Bourne (1887) in Madras, who worked with a large species of Indian scorpion and showed that not only was the so-called suicide a myth, but that the scorpion is immune to scorpion venom.

Metchnikoff (1901, p. 344) confirmed this, and in addition made

the very interesting observation that the blood of the scorpion is distinctly anti-toxic as regards scorpion venom, and to so great a degree that one-tenth of a cubic centimetre ('1 c.c.) of scorpion blood is sufficient to protect a mouse against a dose of the venom which would cause death in half an hour.

This anti-toxic power of the blood he found to be the same in both *Scorpio afer* and *Androctonus*.

He points out that this is the only example known of an anti-toxin occurring in an invertebrate, and discusses the question as to whether the anti-toxic power of the blood must be regarded as natural and innate or as having been acquired during life.

Metchnikoff (1901, p. 384) also showed that the blood of the crayfish, when injected into mice, protects the latter against doses of scorpion venom given from a few minutes to an hour later; a most interesting fact, seeing that the crayfish itself is highly susceptible to the venom.

Kobert (1902) refers to the scorpion poison as giving rise to an anti-body when injected, but gives no references.

Calmette (1905, 1907) found the venom of the *Scorpio occitanus* was neutralised by the serum of animals immunised against snake poison, and states that Metchnikoff has verified the fact.

Nicolle and Catouillard (cited by Calmette, 1907, p. 293) on the other hand found that the same anti-venom was inactive against the venom of the scorpion occurring in Tunis. (*Heterometrus maurus*.)

Talaat (1904) working under Ruffer at the Medical School in Cairo immunised three goats with scorpion poison and found that the serum of these animals was capable of neutralising the venom.

#### *Preparation of anti-toxin.*

For this purpose horses were employed. The horse is very highly susceptible to scorpion venom, although the degree of susceptibility varies considerably in different individuals. One horse which received the poison of only one scorpion subcutaneously, showed very marked symptoms. The site of the injection was obviously very painful, and the animal stood with the tail arched, and the hind legs were often stretched out stiffly, presenting in fact the typical picture of an early case of tetanus.

For this reason in commencing the immunisation, the venom was usually mixed with Gram's solution of iodine, this mixture causes no symptoms, but appears to create a certain degree of immunity, so that later when the venom is given alone it is better supported.

The amount of iodine in the mixture was gradually diminished, and after a few injections a small dose of the venom without iodine was given. The injections were repeated at intervals of about a week, gradually increasing the dose, until about 500 c.c. (corresponding to the venom of 500 scorpions) was reached. The animals were then allowed an interval of rest from 14 days to a month, and then bled. The whole process of immunisation is exactly similar to the immunisation of the horse against tetanus toxin. The injections were always given intramuscularly.

When large doses were given, they always caused very severe symptoms, viz.:—pain at the site of inoculation, profuse salivation, repeated straining, with passage of urine and faeces, and great restlessness and sweating. The symptoms come on a few minutes after the injection, and last several hours. Next morning the animal, as a rule, looks perfectly well, and shows nothing but some swelling at the site of the inoculation, or in the brisket.

The horses were weighed once a month, so as to have some check on their general condition; after bleeding the blood was whipped, centrifuged, and filtered through a Berkefeld filter after the addition of 0·5% carbolic acid. (Ehrlich's mixture.)

In order to obtain a good yield of serum it was unfortunately found necessary to whip the blood, as in the case of the first two horses immunised, on allowing the blood to clot, there was practically no separation of serum. Whether this was due to the treatment of the animals, or was merely an individual peculiarity of the two animals in question was not settled.

#### *Testing the serum.*

When these experiments were begun there was rather a scarcity of guinea-pigs in the laboratory, so the earlier tests were made on pigeons. The serum and venom were mixed and injected into the muscles of the breast. This method gave fairly good results, but there was always a risk of some of the mixture exuding out of the needle puncture: therefore as soon as guinea-pigs were available in sufficient numbers they were employed exclusively. Both subcutaneous and intraperitoneal injections were tried, but the latter were found to give more constant results.

The strength of the serum obtained so far from two horses whose immunisation is fairly well advanced is practically the same in both

animals; 2 c.c. of the serum being required to neutralise 1 c.c. of the scorpion extract (corresponding to the venom of one scorpion), i.e. about 10 minimal lethal doses for a guinea-pig of from 500 to 600 grammes, when given intraperitoneally.

As 1 c.c. of scorpion extract represents approximately 2 milligrammes of the venom, one cubic centimetre of the serum neutralises 1 milligramme of the venom. It is hoped however, that by continuing the immunisation, a higher value will be obtained.

As the toxic value of scorpion venom is approximately the same as that of cobra venom (Wilson) it is interesting to note that the anti-toxic value of anti-scorpion serum is of the same order of magnitude as in the case of anti-cobra sera, where 1 c.c. was found by Lamb and Hanna (1902) to be capable of neutralising about 0·7 mgm. of the pure cobra venom.

#### *Prophylactic action of the serum.*

The serum has a powerful prophylactic action. A guinea-pig weighing 690 grammes received 6 c.c. of somewhat weak anti-scorpion serum intraperitoneally, and a quarter of an hour later 9·5 c.c. of a scorpion extract (corresponding to 3 M.L.D.), the animal remained well and showed no symptoms.

#### *Curative action of the serum.*

The curative action of the serum naturally varies enormously with the method of administration of the venom and the serum. If the venom is given intraperitoneally and followed after an interval of half an hour by an injection of serum also intraperitoneally, the results are most striking, as is shown in the following table.

#### *Curative action of scorpion anti-venom on guinea-pigs.*

Venom given intraperitoneally followed by the anti-serum also intraperitoneally, half an hour later.

Animals receiving serum					Controls (no serum)				
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result		Weight of guinea-pig	Venom in c.c.	Anti- serum	Result	
460 grs.	0·20	2·0	Recovered		460 grs.	0·20	—	Died in 4 hours.	
510	0·25	2·0	Recovered		540	0·25	—	Died in 3 h. 20 m.	
500	0·30	2·0	Recovered		480	0·30	—	Died in 1 hour.	

Thus the guinea-pig which has received a dose sufficient to cause death in an hour may be saved by an intraperitoneal injection of serum given half an hour after the venom, i.e. at a time when it is seriously ill.

If the venom is given subcutaneously, followed by the serum also given subcutaneously after an interval of half an hour, the results are not so striking. The animal can be saved from the effects of one certain minimal lethal dose, but if larger quantities of the venom are given, there is only a retardation of the time of death.

This is shown in the following table :—

*Curative action of scorpion anti-venom on guinea-pigs.*

Venom given subcutaneously, followed by anti-serum also subcutaneously, half an hour later.

Animals receiving serum				Controls (no serum)			
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result	Weight of guinea-pig	Venom in c.c.	Anti-serum	Result
530 grs.	0·15	2·0	Recovered	520 grs.	0·15	—	Died in 7 h. 30 m.
465	0·20	2·0	Died in 5 h. 25 m.	470	0·20	—	Died in 3 h. 30 m.
480	0·30	2·0	Died in 2 h. 45 m.	500	0·30	—	Died in 47 mins.

It was thought that possibly better results might be obtained by giving the serum intraperitoneally, but this method did not show any marked difference in the results, as is shown below :—

*Curative action of scorpion anti-venom on guinea-pigs.*

Venom given subcutaneously, followed by the anti-serum given intraperitoneally, half an hour later.

Animals receiving serum				Controls (no serum)			
Weight of guinea-pig	Venom in c.c.	Anti-serum in c.c.	Result	Weight of guinea-pig	Venom in c.c.	Anti-serum	Result
530 grs.	0·15	2·0	Recovered	530 grs.	0·15	—	Died in 5 h. 30 m.
430	0·20	2·0	Died in 9 hrs.	470	0·20	—	Died in 2 h. 30 m.

It should be noted that these experiments were made with the serum of horses which had not advanced very far in their immunisation, so that their serum was not so powerful as that obtained later, the anti-toxic power of which was at least double that of the serum used for these tests.

*Action of heat on scorpion anti-toxin.*

Scorpion anti-toxin is somewhat resistant to heat, and is not destroyed by heating to 70° C. for 10 minutes.

*Connection between scorpion venom and snake venom.*

Calmette working with scorpions from Tunis and Egypt, found that their venom was neutralised by anti-cobra serum; 3 c.c. of the serum neutralising 1 mgm. of the dried venom; a dose which when mixed with normal horse serum, killed a control guinea-pig. These experiments have been repeated by the writer, using the mixed venom from Upper Egypt scorpions, and Calmette's snake venom; three series of experiments were made on guinea-pigs, but in no case could any protective effect be noted.

*Use of the serum in man.*

During the past summer a certain quantity of anti-scorpion serum has been issued to the government medical officers in Cairo, and to the hospitals of Upper Egypt for the treatment of cases of scorpion sting, and a number of reports on the subject have been received. The number of cases, however, concerning which full details are available is not yet sufficient to allow of any conclusions as to the effect on the mortality, particularly, as it is very difficult to obtain any reliable statistics as to the percentage of deaths in untreated cases.

Out of 23 cases in the town of Cairo which were treated with serum, only one death occurred. This was in a child two years old, who was not seen until two hours after having been stung. The child then received 5 c.c. of serum, but unfortunately the only serum available at the moment was a somewhat weak one, over a year old.

The general impression gained by those who have used the serum is very favourable, and almost all the reports note a very striking effect on the severe pain of the sting.

*Conclusions.*

1. The immunisation of suitable animals with scorpion venom gives rise to the production of an anti-venom.
2. This anti-venom is capable of neutralising the venom when mixed with it *in vitro*, and also acts both prophylactically and curatively in animals.

3. The venom is not fixed by the central nervous system, as is the case with tetanus toxin.
4. Calmette's anti-venine could not be shown to have any neutralising effect on the venom used.
5. Employed curatively in man, the serum appears to have a very marked effect on the intense pain following the sting.

### REFERENCES.

- BERT, PAUL (1885). Venin du scorpion. *Soc. de Biol.* p. 574.
- BOURNE (1887). The reputed suicide of scorpions. *Proc. Roy. Soc.* XLII. p. 17.
- CALMETTE (1895). Contribution à l'étude des venins, des toxines et des serums antitoxiques. *Ann. de l'Inst. Pasteur*, p. 232.
- (16. VI. 1902). Sur l'action hémolytique du venin de cobra. *Compt. rend. Acad. d. Sci.* p. 1446.
- (1907). *Les Venins*, Paris, p. 293.
- FLEXNER and NOGUCHI (1902). Snake venom in relation to haemolysis, bacteriolysis and toxicity. *Journ. of Exper. Med.* p. 277.
- JOYEUX-LAFFUIE (6. XI. 1882). Appareil venimeux et venin de Scorpion. *Thèse de Docteur et Sciences*, et *Comptes rendus Acad. Sci.* p. 866.
- KOBERT (1902). *Lehrbuch der Intoxication*, p. 217.
- KYES and SACHS (1903). Zur Kenntnis der Cobragift activirenden Substanzen. *Berl. klin. Wochenschr.* pp. 21, 57 and 82.
- LAMB and HANNA (1902). Standardisation of Calmette's anti-venomous serum with pure cobra venom: the deterioration of this serum through keeping in India. *Scientific memoirs of the Officers of the Medical and Sanitary Departments of the Government of India*, No. 1 (New series).
- METCHNIKOFF (1901). *L'Immunité*, Paris, p. 344.
- SCHINZ (1891). Deutsch Süd-West Afrika, Oldenburg, p. 396.
- TALAAT (1904). *Comptes rendus du Premier Congrès Égyptien de Méd.*
- VALENTIN (1876). Ueber die Giftwirkung des Nord Afrikanischen Skorpiones. *Zeitschr. f. Biol.* Vol. XII. p. 170.
- WILSON (1904). On the venom of the scorpion. *Records of the Egyptian Government School of Medicine*, Vol. II.

## FURTHER OBSERVATIONS ON THE DIFFERENTIATION OF LACTOSE-FERMENTING BACILLI, WITH SPECIAL REFERENCE TO THOSE OF INTESTINAL ORIGIN.

By ALFRED MacCONKEY, M.B., D.P.H.

*Bacteriologist in charge, Serum Department, Lister Institute, Elstree, Herts.*

IN a previous paper (1906) I entered a plea for the use of certain tests in the bacteriological examination of waters and food stuffs as it seemed we should thus be enabled to better differentiate the lactose-fermenting bacilli. Such differentiation seems necessary because of the importance attached to *B. coli* as an indicator of faecal contamination, and because of the differences of opinion which exist as to the characters which justify us in identifying a bacillus as *B. coli*.

Thus according to Savage (1906, p. 77) the English Committee appointed to consider the Standardisation of Methods for the Bacterioscopic Examination of Water defined *B. coli* as :

"A small, motile, non-sporing bacillus, growing at 37° C. as well as at room temperature. The motility is well observed in a young culture in a fluid glucose medium. It is decolorised by Gram's method of staining. It never liquefies gelatine, and the gelatine cultures should be kept at least 10 days in order to exclude a liquefying bacillus. It forms smooth thin surface growths and colonies on gelatine, non-corrugated, growing well to the bottom of the stab ( facultative anaerobe). It produces permanent acidity in milk, which is clotted within 7 days at 37° C. It ferments glucose and lactose, with the production of both, acid and gas. The typical *bacillus coli* must conform to the above description and tests. It generally also forms indole, gives a thick yellowish brown growth on potato (greatly dependent on the character of the potato), sometimes ferments saccharose (about 50%), changes neutral red (Grübler's) and reduces nitrates, and half the gas produced by it from glucose is absorbable by KOH; and these tests, if time and opportunity permit, may be performed in addition to the foregoing";

and according to Prescott and Winslow (1908, p. 103) the Committee on Standard Methods of Water Analysis of the American Public Health

Association in 1905 drew the following set of diagnostic characters for *B. coli*:

- "(1) Typical morphology—non-sporing bacillus, relatively small and often quite thick.
- (2) Motility—when a young broth or gelatine culture is examined.
- (3) Fermentation of dextrose broth, with the formation of about 50 % of gas, of which about one-third ( $\text{CO}_2$ ) is absorbed by a 2 % solution of sodium hydrate.
- (4) Coagulation of milk, with the production of acid, in 48 hours or more at 37° C., either spontaneously or upon boiling.
- (5) Non-liquefaction of gelatine.
- (6) Production of indole in peptone solution.
- (7) Reduction of nitrates."

These two descriptions differ in the importance they attach to the various characters: the American Committee insisting upon the indole and nitrate tests, while the English Committee consider that it is only necessary to perform these tests "if time and opportunity permit." The characters about which they appear to be in accord are: Morphology, motility, the fermentation of dextrose and lactose, the production of acid and clot in milk and the non-liquefaction of gelatine. But even these few have not all been accepted as absolutely necessary; for Prescott and Winslow (1906, p. 104) and Savage (1906, pp. 154 and 157) think that motility is not always present and that its absence is of no significance. There are also differences of opinion with regard to the value of the appearance of the growth on gelatine and on agar.

It seems therefore advisable to go into the question of the weight which each character should be allowed to carry.

#### TESTS IN COMMON USE FOR DIFFERENTIATION PURPOSES.

##### *Morphology.*

*B. coli* is described as a short bacillus with rounded ends but all sizes may be seen in the same culture.

Buxton (1902, p. 201), speaking of the group of organisms intermediate between *B. coli* and *B. typhosus*, says "Morphologically the intermediates cannot be distinguished among themselves nor with any degree of certainty from *B. coli communis* or *B. typhosus*." Durham (1900—1901, p. 354) says "Speaking generally, morphological characters are not of much value for subdivision of these bacteria" (*B. typhosus*, *B. coli*, etc.). Horrocks (1903, p. 369) describes the morphology of his six groups in the same terms. As his groups include *B. coli*, *B. lactis aerogenes* and

others it is obvious he did not find morphology of much differential value. Barber's (1907) studies "On Heredity in certain micro-organisms" also show how much *B. coli* can vary in its morphology. I have several times examined carefully 50—100 film preparations, one after the other, and tried to classify them according to the size and shape of the bacilli, but I have never yet succeeded in separating even one variety from all the others. I am consequently of opinion that no one would reject an organism as *B. coli* simply on account of an "atypical" morphology.

#### *Motility.*

It is very difficult to arrive at a conclusion with regard to this character. There is no doubt that an organism may be actively motile in a young (say a 6 hours' broth) culture and yet be quite immobile when examined in a drop of the same culture 12—18 hours later. Sometimes dilution has the effect of starting movements in an apparently non-motile culture. An organism may also be motile when grown at 37° C. or vice versa. One would then be inclined to say that this character varies so much that it can carry no weight one way or the other; and yet no one would accept a motile bacillus as *B. lactis aerogenes* or *B. pneumoniae* (Friedländer). If we allow a value to this character in one case can we consistently deny it a value in a closely allied case. May it not be simply want of knowledge which prevents us forming a just appreciation of it, and would it not be as well to observe this character in each case until we can form a definite opinion about it. A drop of a 6 hours' broth culture placed on an ordinary slide without coverslip and examined, under dark ground illumination, with a  $\frac{1}{2}$  inch objective and a  $\times 8$  eyepiece will give an excellent idea of the power of movement possessed by an organism. The drop can then be spread, dried and stained in the ordinary way. It seems a pity not to make this observation when it entails so little labour and may eventually prove a necessity.

#### *Broth, bloodserum, agar, potato.*

The general opinion is that these media have no value for differentiation purposes.

#### *Litmus milk.*

Almost every lactose-fermenter I have tested has produced acid and clot in milk when grown for a sufficient time. All have produced

enough acid to cause coagulation on boiling the milk. This medium is used in routine work not for the investigation of enzyme action but merely to observe the production of acid from lactose. Therefore when a pure lactose medium is used it is unnecessary to use milk as well. If milk be used it must according to Biffi (1906) be sterilised always at the same temperature as many bacteria coagulate it when sterilised at 100° C. which will not coagulate it when sterilised at a higher temperature.

#### *Production of acid in litmus whey.*

This test has not come into general use, and rightly so in my opinion, as the small amount of information obtained from it is far from being commensurate with the trouble entailed in the production of the medium and the performance of the test.

#### *Production of fluorescence in neutral red media.*

So many organisms give this reaction that the tendency is not to lay any stress upon it.

#### *Reduction of nitrites.*

This also appears to be a property common to a large number of organisms and no differential value is attached to it. (Savage, 1906, p. 80—Horrocks, 1903, p. 364—Gruber, Th. 1906, p. 656.) I tested some 70 lactose fermenters and non-lactose fermenters and found that all produced nitrites from nitrates as evidenced by Ilosvay's reagent.

#### *Nutrient gelatine.*

The appearance of slope or stab cultures depends so much upon the quantity and kind of material used for inoculation that it is difficult to understand how these can be preferred to plate cultures. In the case of the latter the presumption is that each colony starts from a single organism and therefore if there is plenty of room for development any characteristic appearance should be more evident in a colony than in a streak or a stab culture. But the colonies of an organism may vary, even on the same plate, and organisms with different characters may grow alike in colony form (Savage, 1904, p. 358, 1906, p. 81—Horrocks, 1903, p. 369—Radzievsky, 1900, p. 369—von Freudenreich, 1904, p. 408—Leichmann, 1899—Löhnis, 1907, pp. 114 and 115—Gruber, 1906, pp. 655

and 719). Klein (1899—1900, p. 373) says "it is not safe from mere appearances on gelatine to regard particular colonies as those of *B. coli* or its varieties. Such colonies cannot without animal experiment be declared not to be the bacillus of pseudo tuberculosis. Moreover they may be neither *B. coli*, nor its varieties, nor the bacillus of pseudo tuberculosis."

Zlatogoroff (1904, p. 520) thinks that the colonies of *B. pestis* on gelatine resemble those of *B. coli*.

Klein and Houston (1899—1900, p. 601) describe a bacillus which gave typical coli-like colonies on gelatine and appeared in every way typical of *B. coli* except that it did not clot milk and liquefied gelatine after some weeks.

Con and Esten (1904 a) after describing the appearance of the colonies of the *B. lactis aerogenes* group on milk-whey-peptone-gelatine (reaction +15 to phenolphthalein) say that this group includes several different species. There are at least four different types. Some of the colonies prove to be *B. coli communis*; some to be cocci instead of rods; others to be *B. lactis aerogenes* except that they do not ferment milk sugar; and in a few cases these colonies prove to be different from any of the foregoing. The same writers in another communication (1904 b) state that *B. lactis aerogenes* and *B. coli communis* produce colonies very similar to each other.

Houston (1904, p. 105) says "The picking out of the coli-like colonies for study in pure culture is after all, even to the expert, a speculative venture."

Longley and Baton (1907) in a paper on the determination of *B. coli* in water say "On account of the insignificant value of the test for liquefaction of gelatine in the examination of the Potomac water.....it may hereafter be omitted from our routine examinations without introducing any appreciable error."

My own experience is that if a pure culture of an organism be plated it is not at all uncommon to find colonies of more than one kind on the same plate, and that it is useless to attach to "typical" gelatine growths more than a confirmatory value. The real value of a gelatine culture lies in the information it affords us of the power of the organism to liquefy gelatine. Even this value is discounted by the fact that some of these organisms liquefy very slowly—too slowly for this character to help us in routine work.

*Production of indole.*

Opinions seem very much divided regarding the value of this test. On going through the literature one very frequently meets with the statement that the power of producing indole is a variable character. Most bacteriologists would agree that a typical *B. coli* should produce indole in peptone water and yet few would state definitely that they would reject a bacillus which differed from a typical *B. coli* only in failing to produce indole. It seems to me that this conflict of opinion in part arises from the use of the sulphuric acid and nitrite test, which without doubt does give varying results. On the other hand, if Ehrlich's test (Böhme, 1905, Marshall, 1907, Steensma, 1906) be used it is rare to find a second test yield a result different to the first. When using this test it is best to use cultures which have been grown for 6—7 days (2 or 3 days is not long enough). Then unless there is a distinct red colour produced it is advisable to shake up the culture with amyl alcohol and extract the colouring matter. On more than one occasion I have noticed a faint pink colour appear after the addition of the test reagents, and I have put down the reaction as positive, but on shaking up with amyl alcohol the pink has entirely disappeared—neither the culture nor the alcohol showing the least trace of it. And on the other hand, cultures which did not show any colour after the addition of the test reagents yielded a pale pink to the amyl alcohol.

If these precautions are taken this test seems to be reliable. One cubic centimetre of each solution is sufficient for a test, and if a red colour appears on the addition of the benzaldehyde it is unnecessary to add the potash.

Cultures in which a deep red colour has appeared often show a peculiar result when treated with amyl alcohol. If such a red culture be shaken up with a small quantity of amyl alcohol some of the red colour will be extracted by the spirit. If the coloured spirit be pipetted off, more alcohol added and the shaking up repeated, more of the colour will be removed, and if this process be repeated several times it will be found that the culture has lost its distinct red and become bluish or violet and sometimes almost black.

*Fermentation tests.*

Glucose and lactose are the only fermentable substances in common use for the differentiation purposes we have in view. *B. coli* is

acknowledged by all to decompose both these sugars with the production of acid and gas. Further tests of this kind seem to be considered unnecessary, and Savage (1906, p. 83) may be said to voice the general opinion when after giving a definition of *B. coli* he says: "Organisms with all the above characters whether they ferment saccharose, dulcit, etc. or not can all be spoken of as *B. coli*."

We have now considered all the tests which are usually employed for the purpose of differentiating *B. coli* from other organisms. It remains to see how far these tests will help us in connection with organisms isolated in the present research.

*Examination of 497 lactose fermenting bacilli isolated from various sources.*

In all 76 samples have been studied, the origin of which was as follows:

Human faeces	20 samples	Rain water	1 samples
Human sputum	9 "	Roof washings	2 "
Human pus	1 "	Oats	1 "
Horse faeces	11 "	Crushed oats	1 "
Calf faeces	7 "	Beans	1 "
Goat faeces	2 "	Ear of corn	1 "
Goose faeces	6 "	Bran	1 "
Pig faeces	1 "	Old hay	1 "
Cesspool sewage	1 "	Malt	1 "
Soil	2 "	Baker's yeast	1 "
Pond water	4 "	Cheese (Coulommier)	1 "

The

"Human pus" was the purulent discharge from the operation wound in a case of appendicitis.

"Pond water" was turbid water taken from a pond in the Institute grounds. It receives the washing of a heavily manured flower border and contains hundreds of gold fish.

"Rain water" was rain water caught in a sterile funnel placed in a sterile flask and left out all one night in a field during rainy weather.

"Roof washings" were rain water collected as it flowed from the rain water pipe of a cow shed in an open field.

"Crushed oats"—"Bran." These were samples taken from the bin in which they were stored for feeding horses.

"Oats"—"Beans" were samples of whole oats and beans collected with little

risk of faecal contamination, and for which I desire to thank Prof. John Percival, Director, Agricultural College, Reading.

"Old hay" was from the interior of a truss cut from a stack two years old.

"Malt" had been kept in a tin box in the laboratory for quite three years.

"Ear of corn" was picked from a hedge bordering a narrow country lane. It hung about 6 feet from the ground. There had been no rain for about 3 weeks, the dust in the lane was about 1 inch deep and the hedges were covered with it. The ear was dropped into a tube of ordinary nutrient bouillon, incubated over night at 37°C. and plated on bile salt lactose agar.

The method of examination was to plate out either direct from the sample or after preliminary incubation in a liquid medium. Where possible bile salt media (MacConkey, 1908) have been employed because of their inhibiting effect upon many organisms of the air, soil and water, the presence of which would have materially increased the amount of labour necessary for the prosecution of the work.

Cultures were made from single colonies, and these presumably pure cultures were subjected to the tests named. That it is necessary to remember that colonies may be composed of more than one variety of organism was emphasized by the fact that some of these cultures were not pure and had to be replated. The results are given in Tables I and II (pp. 94, 95).

A word of explanation is necessary with regard to the numbers attached to the bacilli in the tables. In previous papers the lactose fermenting bacilli were divided into 4 groups according to the fermentative action on saccharose and dulcit. Thus:

Group I contained bacilli which were saccharose — dulcit —

”	II	”	”	”	”	”	—	”	+
”	III	”	”	”	”	”	+	”	+
”	IV	”	”	”	”	”	+	”	—

If we add, as further tests, the action on adonit and inulin the presence or absence of motility, of indole and of Vosges and Proskauer's reaction, it is possible to form 128 combinations, or to put it another way, we might isolate 128 varieties of lactose fermenting bacilli. But only about  $\frac{1}{4}$  of this number have been met with, and so in order to allow for the correct placing of other bacilli 32 numbers have been assigned to each group.

If we study Table I in the light of what has gone before we note that

- (1) all these bacilli ferment lactose with the production of acid and gas,

*Lactose-fermenting Bacilli*

TABLE I. Characters of the bacilli isolated.

No.	1	2 *B. acidilactici* (Filippe)	3 *B. levens*	4 *B. Griththal*, *B. sulcatus gaso-* *formans*, *B. castellus*	5 *B. resticulosus*	6	7	8 *B. coli mutabilis* (Massimi)	9 *B. coli communis*, *B. caricida*	10 *B. Schafferi\**	11 *B. oxytocsus perticusos*	12 *B. rhinocleroma*, *B. Friedländer*	13 *B. neapolitanus*	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	8010	8011	8012	8013	8014	8015	8016	8017	8018	8019	8020	8021	8022	8023	8024	8025	8026	8027	8028	8029	8030	8031	8032	8033	8034	8035	8036	8037	8038	8039	8040	8041	8042	8043	8044	8045	8046	8047	8048	8049	8050	8051	8052	8053	8054	8055	8056	8057	8058	8059	8060	8061	8062	8063	8064	8065	8066	8067	8068	8069	8070	8071	8072	8073	8074	8075	8076	8077	8078	8079	8080	8081	8082	8083	8084	8085	8086	8087	8088	8089	8090	8091	8092	8093	8094	8095	8096	8097	8098	8099	80100	80101	80102	80103	80104	80105	80106	80107	80108	80109	80110	80111	80112	80113	80114	80115	80116	80117	80118	80119	80120	80121	80122	80123	80124	80125	80126	80127	80128	80129	80130	80131	80132	80133	80134	80135	80136	80137	80138	80139	80140	80141	80142	80143	80144	80145	80146	80147	80148	80149	80150	80151	80152	80153	80154	80155	80156	80157	80158	80159	80160	80161	80162	80163	80164	80165	80166	80167	80168	80169	80170	80171	80172	80173	80174	80175	80176	80177	80178	80179	80180	80181	80182	80183	80184	80185	80186	80187	80188	80189	80190	80191	80192	80193	80194	80195	80196	80197	80198	80199	80200	80201	80202	80203	80204	80205	80206	80207	80208	80209	80210	80211	80212	80213	80214	80215	80216	80217	80218	80219	80220	80221	80222	80223	80224	80225	80226	80227	80228	80229	80230	80231	80232	80233	80234	80235	80236	80237	80238	80239	80240	80241	80242	80243	80244	80245	80246	80247	80248	80249	80250	80251	80252	80253	80254	80255	80256	80257	80258	80259	80260	80261	80262	80263	80264	80265	80266	80267	80268	80269	80270	80271	80272	80273	80274	80275	80276	80277	80278	80279	80280	80281	80282	80283	80284	80285	80286	80287	80288	80289	80290	80291	80292	80293	80294	80295	80296	80297	80298	80299	80300	80301	80302	80303	80304	80305	80306	80307	80308	80309	80310	80311	80312	80313	80314	80315	80316	80317	80318	80319	80320	80321	80322	80323	80324	80325	80326	80327	80328	80329	80330	80331	80332	80333	80334	80335	80336	80337	80338	80339	80340	80341	80342	80343	80344	80345	80346	80347	80348	80349	80350	80351	80352	80353	80354	80355	80356	80357	80358	80359	80360	80361	80362	80363	80364	80365	80366	80367	80368	80369	80370	80371	80372	80373	80374	80375	80376	80377	80378	80379	80380	80381	80382	80383	80384	80385	80386	80387	80388	80389	80390	80391	80392	80393	80394	80395	80396	80397	80398	80399	80400	80401	80402	80403	80404	80405	80406	80407	80408	80409	80410	80411	80412	80413	80414	80415	80416	80417	80418	80419	80420	80421	80422	80423	80424	80425	80426	80427	80428	80429	80430	80431	80432	80433	80434	80435	80436	80437	80438	80439	80440	80441	80442	80443	80444	80445	80446	80447	80448	80449	80450	80451	80452	80453	80454	80455	80456	80457	80458	80459	80460	80461	80462	80463	80464	80465	80466	80467	80468	80469	80470	80471	80472	80473	80474	80475	80476	80477	80478	80479	80480	80481	80482	80483	80484	80485	80486	80487	80488	80489	80490	80491	80492	80493	80494	80495	80496	80497	80498	80499	80500	80501	80502	80503	80504	80505	80506	80507	80508	80509	80510	80511	80512	80513	80514	80515	80516	80517	80518	80519	80520	80521	80522	80523	80524	80525	80526	80527	80528	80529	80530	80531	80532	80533	80534	80535	80536	80537	80538	80539	80540	80541	80542	80543	80544	80545	80546	80547	80548	80549	80550	80551	80552	80553	80554	80555	80556	80557	80558	80559	80560	80561	80562	80563	80564	80565	80566	80567	80568	80569	80570	80571	80572	80573	80574	80575	80576	80577	80578	80579	80580	80581	80582	80583	80584	80585	80586	80587	80588	80589	8

TABLE II. *Distribution of the bacilli isolated.*

No.	2	<i>B. acidi lactici</i>	21
"	3	<i>B. levanus</i>	1
"	4	<i>B. Grünenthal</i> , <i>B. sulcatus gasiformis</i> , <i>B. castellus</i>	0
"	5	<i>B. vestitus</i>	18
"	6		
"	7	<i>B. coli mutabilis</i> (Massini)	
"	8		
"	9		
"	10		
"	11		
"	12		
"	13		
"	14		
"	15		
"	16		
"	17		
"	18		
"	19		
"	20		
"	21		
"	22		
"	23		
"	24		
"	25		
"	26		
"	27		
"	28		
"	29		
"	30		
"	31		
"	32		
"	33		
"	34		
"	35		
"	36		
"	37		
"	38		
"	39		
"	40		
"	41		
"	42		
"	43		
"	44		
"	45		
"	46		
"	47		
"	48		
"	49		
"	50		
"	51		
"	52		
"	53		
"	54		
"	55		
"	56		
"	57		
"	58		
"	59		
"	60		
"	61		
"	62		
"	63		
"	64		
"	65		
"	66		
"	67		
"	68		
"	69		
"	70		
"	71		
"	72		
"	73		
"	74		
"	75		
"	76		
"	77		
"	78		
"	79		
"	80		
"	81		
"	82		
"	83		
"	84		
"	85		
"	86		
"	87		
"	88		
"	89		
"	90		
"	91		
"	92		
"	93		
"	94		
"	95		
"	96		
"	97		
"	98		
"	99		
"	100		
"	101		
"	102		
"	103		
"	104		
"	105		
"	106		
"	107		
"	108		
"	109		

+ Yellowish.

\* Yellow liqueur.

- (2) they produce acid and clotting in milk,
- (3) the majority do not liquefy gelatine, and those which do so liquefy so slowly that they would in routine work be classed as non-liquefiers,
- (4) all are Gram-negative,
- (5) the motility and indole tests are positive in some cases and negative in others,
- (6) in all cases which have been tested nitrates were reduced to nitrites.

The morphology is not noted in the Table, because it proved not to be of any differential value.

Now, according to the tests usually employed, all these bacilli would be classed as *B. coli*. But if we kept them long enough we should find that in several cases gelatine was liquefied and we should be in the position of having classified a liquefying organism as *B. coli*, though all bacteriologists are agreed that *B. coli* is not a liquefying organism.

It has been suggested that the error from this cause is so small that it may be neglected, but an enumeration of the liquefiers in Table II shows that the error would be more than 10 % and therefore cannot be ignored. It follows that the present methods of differentiation are not adequate and that a change is necessary.

In a former paper I proposed that we should omit the observation of the (1) characters of the growth on gelatine, (2) action on milk, (3) action on glucose, (4) action on neutral red, and (5) the indole test, and that we should substitute for them (1) the action on dulcitol, (2) the action on adonit, (3) the action on inulin and (4) Vosges and Proskauer's reaction.

Further experience has only confirmed me in this opinion except as regards the indole test and Vosges and Proskauer's reaction. With regard to the fermentation reactions it is necessary to remember that some bacilli act slowly and that, in consequence, we may be premature if we draw conclusions after the cultures have been incubated for only 24, 48 or even 72 hours. I have changed my opinion of the indole test because an extensive use of Ehrlich's reaction has shown me that this test cannot be omitted with safety.

#### *Vosges and Proskauer's reaction.*

I have used this reaction for some years, and it seemed to be reliable and valuable until at the end of 1907 I isolated organisms which gave sometimes a negative or doubtful reaction and sometimes a positive one.

Such results have weakened my faith in this test and yet I cannot say it is of no value as it certainly is not given by every organism. For instance, in 1905 I isolated from an ear of corn 11 lactose fermenting bacilli of which 10 agreed as regards motility and fermentation reactions. These differed in that 5 liquefied gelatine, gave Vosges and Proskauer's reaction and did not produce indole, while the other 5 did not liquefy gelatine, did not give Vosges and Proskauer's reaction but did produce indole. These cultures were kept and tested from time to time and always gave identical results until September 1908 when one of the non-indole-producers gave a positive indole reaction. This culture was at once plated and 6 colonies were subcultured and worked through the various tests. All were motile and all gave the same fermentation reactions as before, but 4 were indole negative and 2 were indole positive. The indole-negative culture gave Vosges and Proskauer's reaction, while the indole-positive ones did not. In this instance Vosges and Proskauer's reaction was of distinct value in confirming the presence of contamination.

No one of these tests is of much value by itself. They must be considered together, each one merely forming one link in the chain of evidence. An example may perhaps make my meaning clearer.

The *B. oxytoccus perniciosus*, No. 65, Table I, would according to the usual tests be classed as a non-motile *B. coli*. But it liquefies gelatine, taking perhaps 8—12 months to liquefy  $\frac{1}{2}$  inch of the medium. So unless we use further tests we should classify this bacillus incorrectly. When however we study the series of tests given in Table I we observe that it

- (1) gives a positive reactive with *all* the fermentable substances used,
- (2) produces indole,
- (3) is non-motile,
- (4) gives Vosges and Proskauer's reaction.

If any reliance is to be placed upon these tests we ought to find that bacilli having these characters liquefy gelatine. This has in my experience proved to be the case.

I isolated from soil several organisms which were *B. oxytoccus perniciosus* so far as was evidenced by fermentation reactions, indole and motility. But they appeared to be non-liquefiers. This led me to try what may be termed the "massive inoculation" test for liquefaction (MacConkey, 1906 a). A positive result proved that these bacilli were in truth *B. oxytoccus perniciosus* and at the same time afforded evidence in favour of the value of this series of reactions.

One might multiply examples of this kind, but there seems to be no real advantage to be gained thereby. It will suffice to say that as regards *B. cloacae* these results have been confirmed by Ferreira, Horta and Paredes (1908).

Coming now to Table II we are struck by the distribution of the bacilli isolated. If all these organisms were the same bacillus we might reasonably expect that they should be distributed fairly evenly throughout the samples. But such is not the case.

The *B. levans* for instance is so rare that it has not been met with once in 497 bacilli, and yet *B. levans* has been stated to be identical with *B. coli* (Papasotiriou, 1902).

On the other hand 7 of the varieties given in Table I claim 87 % and 3 varieties 62 % of the 178 bacilli obtained from human faeces, etc., and of the 154 organisms with an origin in animal faeces 68 % belong to 2 varieties and 46 % to a single variety. If we consider the 497 bacilli as one whole we find that 54·9 % of them belong to one or other of 3 varieties. If all these bacilli are *B. coli* we must allow that certain varieties of this organism are very common in faeces and material exposed to faecal contamination, while certain other varieties are rare in faeces and more common in other materials.

This suggests that the term *B. coli* as at present used is not a happy one, it is too comprehensive, and it would be better to avoid using it. There can be little doubt that all these bacilli have already been isolated and partially described under some name, but I have found it impossible to obtain more than a few named cultures. For the others we must be content to use numbers until we find out the names by which they should properly be known.

I think I have now brought forward sufficient evidence to justify me in once more urging the adoption in routine work of the series of tests recommended in this paper.

Only two further points need be touched upon. Firstly objection may be taken to my classification on the ground that the fermentation reactions are not stable. Some workers, Klotz (1906), Reavis (1908), Twort (1907), seem to consider that these reactions are too inconstant to be of use in classification, but the work of Savage (1906), Horrocks (1903), p. 370, MacConkey (1905, p. 356, 1906 b, p. 399), Villinger (1894), and Benczur (1908), is evidence in favour of the reliability of these tests.

Nor must it be forgotten that this very kind of reaction is considered of great value in the differentiation of *B. coli*, *B. typhosus* and *B. enteritidis* (Gaertner). If they are looked upon as reliable enough for this purpose why should they not be equally of value in separating *B.*

*coli* from *B. lactis aerogenes* and similar organisms. To say that an organism which does not decompose lactose is not a *B. coli* and then to say that the decomposition or not of some other substance, say dulcitol, is of no value in separating *B. coli* from another lactose fermenter is, to say the least, inconsistent.

But there is no need to labour this point. From a practical standpoint it matters not whether these organisms are different bacilli or one bacillus in various guises. If organisms isolated from a certain material (*A*) give in the majority of cases a certain series of reactions ( $a_1, a_2, a_3$ ), and if organisms isolated from some other material (*B*) give in the majority of instances a different series of reactions ( $b_1, b_2, b_3$ ) then we would be justified in associating the series ( $a_1, a_2, a_3$ ) with the material (*A*) and the series ( $b_1, b_2, b_3$ ) with the material (*B*); and if we found both the ( $a_1, a_2, a_3$ ) series and the ( $b_1, b_2, b_3$ ) series given by organisms isolated from (*B*) we could presume that some of (*A*) had become mixed with (*B*). It might be that both series of reactions were given by the same organism and that the difference was simply the effect of environment. This would not affect the conclusion. It would only show that the organism had not been long enough in (*B*) to have its ( $a_1, a_2, a_3$ ) series altered by the changed environment. Secondly it may be asked : "Why limit oneself to these few reactions ? Why not use more fermentable substances and have still better differentiation?" I can only answer that I have not found any real advantage in so doing.

We all of us always wish to identify organisms as accurately as possible, in as short a time as possible, and with as little trouble as possible. This desire with regard to time and trouble is intensified when it is a question of routine work. The exigencies of this class of work must always be borne in mind. With this end in view I have given a thorough trial to the following substances :

Glucose—laevulose—galactose—lactose—maltose—mannose—arabinose—raffinose—saccharose—mannit—dulcitol—adonit—quercit—erythrit—inosit—sorbit—glycerine—arbutin—salicin—amygdalin— $\alpha$ -methyl-glucoside—inulin—dextrin—and starch;

and I have come to the conclusion that for the purpose of differentiating the lactose fermenters we can be content to use lactose, saccharose, dulcitol, adonit, inulin, inositol, and possibly mannit<sup>1</sup>. It does not seem worth while using any of the other substances mentioned : for quercit

<sup>1</sup> It may become necessary to use mannit because there is a bacillus which ferments glucose and lactose but not mannit.

and erythrit remained unaffected when tested with about 100 organisms taken at random; and the others did not afford any more information than is to be gained by using the substances mentioned. These together with the indole test, the observation of motility and perhaps Vosges and Proskauer's reaction will enable us to travel a fair distance on the road to our goal.

The method of procedure suggested is that a sloped agar tube should be inoculated from a single colony on a plate, the growth being rubbed all over the surface of the medium and in the water of condensation. After 4—6 hours' growth at 37° C. a drop of the condensation water can be examined to ascertain the presence or absence of motility. After 24 hours' incubation at 37° C. a good loopful of the growth is put into tubes of gelatine, lactose, saccharose, dulcitol, adonit and inulin. The agar tube is returned to the incubator, together with the rest of the tubes, and is used later for the indol test. An inosit tube and a glucose tube (for Vosges and Proskauer's reaction) may be inoculated at the same time as the others, or these two may be used as confirmatory tests. Vosges and Proskauer's reaction may be tested for at the end of 4 days. The other tubes should be kept under observation as long as there is no change in the reaction of the medium.

In the course of this work several bacilli have been isolated which have not been included in the results given above. All these bacilli will be found in the subjoined Table III which gives their characters.

#### CONCLUSIONS.

It has been shown that the tests at present in general use do not allow us to differentiate adequately the lactose fermenting bacilli from each other. It has also been shown that by the substitution and addition of certain other tests we shall gain in accuracy with little increase in labour, and that we shall thus have a fair prospect of being able to pick out those organisms which are most closely associated with faeces and put the bacteriological examination of water supplies upon a firmer basis than that upon which it stands at present.

#### *Note.*

While this communication was in the press my attention was directed to a paper by Bergey and Deehan on "The Colon-aerogenes

TABLE III.

\* It has been my experience that in cases of this kind (where an organism produces acid and gas in one medium and apparently only acid in another medium), under proper subinoculation the organism will produce gas also in the second medium. The last organism in the series, *Candida*, produces acid only, no gas.

acid in another medium) under proper subcultivation the organism will produce gas also in the second medium. But where an organism produces only acid in several media, e.g. the 3rd organism in Table III, then I have never succeeded in inducing it to produce gas in any one of them.

Group of Bacteria" in the *Journal of Medical Research*, Vol. xix. No. 1 (July, 1908).

These workers examined 50 samples of milk, 1 sample of Kefir and 8 samples of sewage. They made use of the tests advocated above and their results cause them to express opinions which are in complete agreement with mine as to the value of these tests. They attach importance to the gas amount and gas ratio. I have not referred to these two tests because they have been shown to be unreliable by Longley and Baton (1907).

### BIBLIOGRAPHY.

- BARBER (1907). On Heredity in certain micro-organisms. *The Kansas University Science Bulletin*, Vol. iv.
- BENCZUR (1908). Kleiner Beitrag zur Frage der Identität des Typhus- und Coli-Bacillus. *Centralbl. f. Bakt. Abt. I. Originale*, Vol. xlvi. Heft 3, pp. 275—277.
- BIFFI (1906). La coagulabilità al calore delle culture in latte come elemento di diagnosi batteriologica. *Soc. medicochirurgica di Bologna*. 16. XII. 1906. *Centralbl. f. Bakt. Abt. I. Referate*, Vol. xl, p. 312.
- BÖRME (1905). Die Anordnung der Ehrlich'schen Indolreaktion für bakteriologische Zwecke. *Centralbl. f. Bakt. Abt. I. Originale*, Vol. xl, pp. 129—133.
- BUXTON (1902). A comparative study of the bacilli intermediate between *B. coli communis* and *B. typhosus*. *Journ. of Med. Research*, Vol. viii. pp. 201—230.
- CONN and ESTEN (1904 a) Qualitative analysis of bacteria in market milk. *Studies from the Rockefeller Institute for Medical Research*, Vol. i.
- CONN and ESTEN (1904 b). The comparative growth of different species of bacteria in normal milk. *Studies from the Rockefeller Institute for Medical Research*, Vol. i.
- DURHAM (1900—1901). Some theoretical considerations upon the nature of agglutinins, together with further observations upon *Bacillus typhi abdominalis*, *Bacillus enteritidis*, *Bacillus coli communis*, *Bacillus lactis aerogenes*, and some other bacilli of allied character. *Journ. of Experiment. Med.* Vol. v. pp. 354—388.
- FERREIRA, HORTA and PAREDES (1908). Recherches sur le *B. coli communis* de l'intestin de l'homme. *Archivos de Real Instituto Bacteriologica Camara Pestana*. Lisboa : Tome II. Fasc. II.
- v. FREUDENREICH (1904). Ueber die Bakterien im Kuheuter und ihre Verteilung in den verschiedenen Partien des Melkens. *Centralbl. f. Bakt. Abt. II. Vol. XIII.* pp. 281 and 407.
- GRUBER (1906). Die beweglichen und unbeweglichen aeroben Gärungserreger in der Milch. *Centralbl. f. Bakt. Abt. II. Vol. XVI.* pp. 654—663, 711—719.
- HORROCKS (1903). The *Bacillus coli communis* considered as an indicator of sewage contamination of water supplies. *Journ. of the Roy. Army Med. Corps*. Vol. i. pp. 362—376.
- HOUSTON (1904). *Fourth Report of the Royal Commission on Sewage Disposal*, Vol. III. p. 105.

- KLEIN (1899—1900). On Pseudotuberculosis, its Pathology and Etiology. *Report of the Medical Officer to the Local Government Board*, pp. 355—384.
- KLEIN and HOUSTON (1899—1900). On bacterioscopic analysis of cereals and food stuffs. *Report of the Medical Officer to the Local Government Board*, pp. 593—604.
- KLOTZ (1906). Temporary alteration of character of an organism belonging to the colon group. *Journ. of Infectious Diseases*. Supplement No. 2, pp. 35—39.
- LEICHMANN (1899). Ueber die Beteiligung des *Bacillus lactis aerogenes* an der freiwilligen Säuerung der Milch. *Centralbl. f. Bakt. Abt. II. Vol. V.* pp. 344—349. Ref. *Kochs Jahresbericht*, 1899, Vol. x. p. 200.
- LÖHNIS (1907). Versuch einer Gruppierung der Milchsäurebakterien. *Centralbl. f. Bakt. Abt. II. Vol. XVIII.* pp. 97 and 149.
- LONGLEY and BATON (1907). Notes on the determination of *B. coli* in water. *Journ. of Infectious Diseases*, Vol. IV. pp. 397—416.
- MACCONKEY (1905). Lactose fermenting bacteria in faeces. *Journ. of Hyg.* Vol. V. pp. 333—379.
- (1906 a). On the liquefaction of gelatine by the *Bacillus cloacae*. *Journ. of Hyg.* Vol. VI. pp. 23—32.
- (1906 b). A contribution to the bacteriology of milk. *Journ. of Hyg.* Vol. VI. pp. 385—407.
- (1908). On bile salt media and their advantages in some bacteriological examinations. *Journ. of Hyg.* Vol. VIII. pp. 322—334.
- MARSHALL (1907). The Para-Dimethyl-Amido-Benzaldehyde Test for Indole. *Journ. of Hyg.* Vol. VII. pp. 581—588.
- PAPASOTIRIU (1902). Untersuchungen über das Vorkommen des *Bakterium coli* in Teig, Mehl und Getreide. *Archiv für Hyg.* Vol. XLI. p. 204.
- PREScott and WINSLOW (1908). *Elements of Water Bacteriology*. New York, 1908.
- RADZIEVSKY (1908). Beitrag zur Kenntnis des *Bakterium coli*. *Zeitschr. f. Hyg.* Vol. XXXIV. pp. 369—453.
- REEVIS (1908). Milk and the physiological properties of coli-form organisms. *Journ. of Path. and Bact.* Vol. XII. pp. 228—232.
- SAVAGE (1904). Gelatine surface colonies of *B. coli communis*. *Journ. of Path. and Bact.* Vol. IX. pp. 347—358.
- (1906). *The bacteriological examination of water supplies*. London, 1906.
- STEEENSMA (1906). Ueber den Nachweis von Indol und die Bildung von Indol vortäuschenden Stoffen in Bakterienkulturen. *Centralbl. f. Bakt. Abt. I. Originale*, Vol. XLI. pp. 295—298.
- TWORT (1907). The fermentation of Glucosides by bacteria of the typhoid-colic group and the acquisition of new fermenting powers by *Bacillus dysenteriae* and other organisms. *Proc. of the Roy. Soc. B.* Vol. LXXIX. p. 329.
- VILLINGER (1894). Ueber die Veränderung einiger Lebenseigenschaften des *Bakterium coli commune* durch äussere Einflüsse. *Archiv f. Hyg.* Vol. XXI. pp. 101—103.
- ZLATOGOROFF (1904). Zur Morphologie und Biologie des Microben der Bubonpest und des Pseudotuberculosisbacillus der Nagetiere. *Centralbl. f. Bakt. Abt. I. Originale*, Vol. XXXVII. pp. 345, 513, 654.

## VARIETIES OF THE MENINGOCOCCUS WITH SPECIAL REFERENCE TO A COMPARISON OF STRAINS FROM EPIDEMIC AND SPORADIC SOURCES.

By JOSEPH A. ARKWRIGHT, M.D.

*Assistant Bacteriologist, Lister Institute of Preventive Medicine.*

THE following paper is based on the results obtained by cultivating and experimenting with strains of Meningococcus from 45 different cases of Meningitis. Twenty-five of these occurred in Epidemic areas and 20 were Sporadic cases occurring in London. The Epidemic strains are referred to throughout this paper as E. 1, E. 2, E. 3, etc., and the London strains (Sporadic) as L. 1, L. 2, L. 3, etc. For most of the Epidemic strains I am indebted to Dr M. Macdonald of the Lightburn Hospital, Shettleston, and to Dr C. B. Ker of the Fever Hospital, Edinburgh. Also I have used strains sent to the Lister Institute by Professor Symmers from Belfast and by Dr Buchanan from Glasgow.

The Sporadic strains are all from London sources and were chiefly isolated from specimens sent to the Lister Institute; for other strains I am indebted to Dr Graham Forbes, Dr F. E. Batten and Miss A. Taylor.

The points to which I have specially directed my attention are

(1) the variability of the Meningococcus and the usual directions which variation takes;

(2) the question of a difference between Sporadic and Epidemic strains;

(3) the occurrence in cases of Meningitis of certain other Gram-negative organisms which have some resemblance to the Meningococcus.

### VARIATIONS.

The differences observed amongst the strains of Meningococcus which I have cultivated have not concerned the morphology nor the staining reactions.

All the strains have been consistently Gram-negative, and they have never grown in chains. With the one exception of a strain which I described in a previous paper (Arkwright, 1908), no growth has taken place at a temperature of 24° C. It has always been easy to make uniform suspensions of young agar cultures in salt solution ('85 %), and no spontaneous clumping has occurred in these suspensions. On the other hand the fermentation reactions in different sugars have not been quite uniform nor constant.

The sugars chiefly employed have been Glucose, Maltose, Laevulose and Cane sugar.

The fermentation of the sugars was tested in fluid media, either weak broth (25 % peptone broth and 75 % peptone water) or 2 % peptone water, ascitic fluid or serum being added to each tube.

On solid media with sugar and litmus added (method of v. Lingelsheim), the cultures were found to give usually a more rapid, but in many instances a very transient, reaction. The red colour changed again to blue in a few hours and the initial acid reaction varied much in its time of appearance with different strains, so that the results had to be noted at different times for different strains. Trautmann and Fromme (1908) also found that this medium for testing the fermentations of sugars gave irregular results of the same kind.

The reactions with sugar broth were not quite uniform, but were almost always complete by the 5th day.

The most constant features of the fermentations have been the formation of acid from Glucose and Maltose but never from Cane sugar. Laevulose was fermented by several strains, acid being produced when the medium contained broth but never when peptone water only was used. This is interesting, as some English observers have found Laevulose to be fermented, though German writers have usually considered the absence of acid-formation from Laevulose to be an important and distinctive characteristic of the Meningococcus.

With regard to Glucose and Maltose, when broth was used, Maltose was fermented most regularly and sometimes first, but when serum-peptone water only was used the Glucose tube most constantly showed the presence of acid and was generally the first to do so.

Several strains at some stage of their artificial culture lacked the power to produce acid from either Glucose or Maltose or (in a few cases) even from both. For instance Strain L. 5 at the earliest testing fermented no sugars and for months fermented only Maltose, but latterly after 10 months' artificial culture fermented both Glucose and Maltose. Strain

E. 2 on the other hand at first produced acid from both Glucose and Maltose but later fermented neither.

Strains L. 13 and E. 3 never fermented any of the sugars.

The change during long artificial culture in the power of the Meningococcus to ferment some particular sugar has been noted by Trautmann and Fromme (1908). The Meningococcus, therefore, shows evidence of mutation as regards its fermentation characters similar to that recorded by Twort (1907) for the *Bacillus typhosus* under special cultural conditions, and by Andrewes and Horder (1906) to a slight extent for Streptococci.

It may be mentioned here that a strain of Gonococcus, which was isolated from the urethral discharge in a case of acute gonorrhoea in an adult, fermented Glucose and Maltose and not Cane sugar, thus in its sugar reactions resembling most strains of Meningococcus. The fermentation of these two sugars has been found to be characteristic of the Gonococcus by Wollstein (1907) and Gurd (1908). Gordon however found that the Gonococcus fermented Glucose and Galactose but not Maltose and Cane sugar, and this I confirmed for one strain.

Table I shows the sugar reactions given by the various strains of Meningococcus, the Sporadic (L.) and Epidemic strains (E.) being placed in different series.

Table II shows the frequency with which variations from the typical sugar reactions occurred among London and Epidemic strains respectively.

It will be apparent that there is not much difference between the Sporadic and Epidemic strains as regards their sugar reactions; Epidemic strains are rather more uniform than the Sporadic.

#### SUMMARY OF FERMENTATION TESTS.

When the tests were made in weak broth with the addition of serum, of 14 Sporadic strains 5 fermented Glucose, Maltose and Laevulose, 6 fermented Glucose and Maltose, 1 Glucose and Laevulose, 1 Maltose only, and 1 no sugars. In three of the strains the reactions were not quite constant.

Of 22 Epidemic strains 12 fermented Glucose, Maltose and Laevulose, 9 Glucose and Maltose and 1 no sugars.

When 2% peptone water with serum was used as a basis for the sugar media, of 7 Sporadic strains 6 fermented Glucose and Maltose and 1

TABLE I.

No. of sporadic strains (L.)	Source	During life or P.M.	Growth at 24°C.	Sugar reactions			
				Broth+serum		2% peptone water+serum	
1	Men.	P. M.	-	-	-	-	-
2	Men.	P. M.	-	-	-	-	-
3	C.-S. Fl.	Life	-	-	-	-	-
4	Men.	P. M.	-	-	-	-	-
5	C.-S. Fl.	Life	-	-	-	-	-
6*	"	"	-	-	-	-	-
7	"	"	-	-	-	-	-
8	"	"	-	-	-	-	-
9 (1)	Blood	P. M.	-	-	-	-	-
9 (2)*	C.-S. Fl.	Life	-	-	-	-	-
10	"	"	-	-	-	-	-
11	"	"	-	-	-	-	-
12	"	"	-	-	-	-	-
13	"	"	-	-	-	-	-
14	"	"	-	-	-	-	-
15*	"	"	-	-	-	-	-
16	"	"	-	-	-	-	-
17	"	"	-	-	-	-	-
18	"	"	-	-	-	-	-
19	"	"	-	-	-	-	-
20	"	"	-	-	-	-	-
21	"	"	-	-	-	-	-
22	"	"	-	-	-	-	-
23*	C.-S. Fl.	Life	+	-	-	-	-
No. of epidemic strains (E.)							
1	C.-S. Fl.	Life	-	-	-	-	-
2	"	"	-	-	-	-	-
3	"	"	-	-	-	-	-
4	"	"	-	-	-	-	-
5	"	"	-	-	-	-	-
6	"	"	-	-	-	-	-
7	"	"	-	-	-	-	-
8	"	"	-	-	-	-	-
9	"	"	-	-	-	-	-
10	"	"	-	-	-	-	-
11			-	-	-	-	-
12	Men.	P. M.	-	-	-	-	-
13	C.-S. Fl.	Life	-	-	-	-	-
14	"	"	-	-	-	-	-
15	"	"	-	-	-	-	-
16	"	"	-	-	-	-	-
17	"	"	-	-	-	-	-
18	"	"	-	-	-	-	-
19	Men.	P. M.	-	-	-	-	-
20	C.-S. Fl.	Life	-	-	-	-	-
21	"	"	-	-	-	-	-
22	"	"	-	-	-	-	-
23	"	"	-	-	-	-	-
24	"	"	-	-	-	-	-
25	"	"	-	-	-	-	-

+ = Acid produced.

- = No acid produced.

± = Acid produced on one occasion and not on another.

S = Slight.

\* These strains of Gram-negative coccus are not Meningococci.

Glucose only. Two of these strains at another time did not ferment any sugars.

Of 13 Epidemic strains 8 fermented Glucose and Maltose, 3 Glucose only and 2 did not ferment any sugars (*vide* Tables II and III).

Tables II and III show the number of strains giving each variety of fermentation.

TABLE II.

*In Serum Broth.*

	Glucose, Maltose & Laevulose	Glucose & Maltose	Glucose	Maltose	Glucose & Laevulose	Maltose & Laevulose	No sugars fermented
14 London strains	5	6 (2)	(1)	1 (1)	1	(1)	1
22 Epidemic strains	12	9	—	—	—	—	1

TABLE III.

*In Serum Peptone Water.*

7 London strains	—	6	1	—	—	—	(2)
18 Epidemic strains	—	8	3 (1)	—	—	—	2

The figures in brackets denote strains which gave different results on other occasions and so appear twice in the Table.

## AGGLUTINATION EXPERIMENTS.

The agglutination experiments were all made by the microscopic method at the temperature of the laboratory and the observations were completed at the end of two hours.

Agglutination tests were made with the serum of a horse which was injected with killed, followed by living, cultures of Meningococcus by Dr MacConkey at Elstree. The injections were partly subcutaneous and partly intravenous. At first only one Sporadic strain (L. 1) was used and later after these injections had been made for a year, one strain of Epidemic Meningococcus (E. 12) was injected into the same horse for a further period of 10 months. Serum "1" was obtained by bleeding the horse after injections of strain L. 1 had been carried on for 12 months. This serum agglutinated the homologous strain L. 1 in dilutions up to 1—500, and several other Sporadic strains in dilutions of 1—100, but only three Epidemic strains were agglutinated by it in dilutions higher than 1—5 and none up to 1—100. It was found, however, that five Sporadic strains were likewise not agglutinated by Serum 1 in higher dilutions than 1—5. The strains which agglutinated best were among

those which had been longest isolated. As however, at least, one Sporadic strain (L. 8) which had been isolated for as long as one year was among those strains which were not agglutinated, the agglutinability cannot be simply the result of long artificial culture.

TABLE IV.

*Microscopic Agglutination.*

Serum 1 = Serum of horse injected with Sporadic strain L. 1 only.

Serum 2 = Serum of same horse after receiving in addition the Epidemic strain E. 12 for 10 months.

Date of exp.	Strains of Meningococcus (London strains)	Serum 1			Serum 2			Normal Serum			Normal salt solution	Date of isolation	
		# 1/5	# 1/25	# 1/100	# 1/500	# 1/25	# 1/100	# 1/500	# 1/2000	# 1/1000	# 1/500		
11/2/08	L. 1	#	#	#	# 1/5								1906
"	L. 5	#	#	#	+	+	-						3/4/06
15/2/08	L. 7	#	#	#	#	+	-						-/2/07
11/2/08	L. 12	#	#	#	#	+							21/5/07
21/3/08	L. 15*	#	-	-	-	-							24/1/08
25/3/08	L. 16	-	-	-	-	-	-						28/2/08
21/3/08	L. 18	#	-	-	-	-	-						11/3/08
"	L. 19	#	-	-	-	-	-						17/3/08
"	L. 20	#	+	-	-	-	-						-/3/07
26/2/08	L. 21	-	-	-	-	-	-						
	L. 22	#	+	-	-	-	-						
15/2/08	L. 8	#	-	-	-	-	#	-	-				1907
	(Epidemic strains)												
15/2/08	E. 2	+	-	-	-	-	#	-	-				30/11/06
"	E. 4	#	#	-	-	-	#	#	#	+	-		18/12/06
14/2/08	E. 5	+	-	-	-	-	#	+	+	+ s	+ s	-	
"	E. 7	+	-	-	-	-	#	+	-	-	-	-	8/2/07
11/2/08	E. 12	#	-	-	-	-	#	+	-	-	-		8/3/07
"	E. 16	-	-	-	-	-	#	+	-	-	-		21/5/07
21/3/08	E. 17	#	+	-	-	-	-	-		#	+	-	
26/3/08	E. 18	#	#	-	-	-	-	-	-	+	-	-	
25/3/08	E. 19	-	-	-	-	-	-	-	-	-	-	-	
26/3/08	E. 21	-	-	-	-	-	-	-	-	-	-	-	
"	E. 22	-	-	-	-	-	-	-	-	-	-	-	-/3/08
27/3/08	E. 23	-	-	-	-	-	-	-	-	-	-	-	-/2/08
26/3/08	E. 24	-	-	-	-	-	-	-	-	-	-	-	1907
27/3/08	E. 25	-	-	-	-	-	-	-	-	-	-	-	1907

\* This strain of Gram-negative coccus is not Meningococcus.

# = Complete agglutination.

+ = Slight

"

# = Incomplete agglutination.

+ s = Very slight

"

The injections of strain E. 12 were carried out on the same horse during the months following the cessation of the strain L. 1 injections. The animal was then bled a second time yielding Serum "2."

This bivalent serum possessed agglutinins for some Epidemic strains, but these could be entirely removed by absorption with the homologous Epidemic strain (E. 12) and partly by absorption with the homologous Sporadic strain (L. 1). The agglutination was only partial however in dilutions above 1—100 for any Epidemic strain.

Serum "3," which came from the same horse at a later date after the injections with E. 12 had been carried on for 10 months, did not give high agglutination with six Epidemic strains, but agglutinated E. 12 (homologous strain) and E. 19 in dilution of 1—500.

Kolle's Anti-Meningococcus serum was used to test the agglutination of 22 strains. This serum agglutinated some Epidemic strains better than any of the Sporadic strains. Of 11 Epidemic strains tested, six were agglutinated in a dilution of the serum of 1—100 or 1—200, whilst of the Sporadic strains tested, only one was agglutinated in a higher dilution than 1—20.

TABLE V.

*Microscopic Agglutination.*

Serum 3 = Serum of same horse which produced Serums 1 and 2, after it had received injections of Epidemic strain E. 12 for 10 months.

Meningococcus London strains	Serum 3				Normal Serum		Normal salt solution
	1/20	1/100	1/500	1/2000	1/20	1/100	
L. 1	##	##	#	+	##	-	-
L. 15*	+	-	-	-	+	-	-
L. 16	-	-	-	-	-	-	-
L. 19	-	-	-	-	-	-	-
L. 23*	##	+	+	-	##	-	+
Epidemic strains							
E. 2	##	-	-	-	+	-	-
E. 7	-	-	-	-	-	-	-
E. 12	##	##	##	-	+	-	-
E. 18	-	-	-	-	-	-	-
E. 19	##	##	##	-	-	-	-
E. 21	-	-	-	-	-	-	-
E. 22	##	+	-	-	+	-	-
E. 23	+	-	-	-	-	-	-
E. 25	+	-	-	-	-	-	-

\* These strains are not Meningococci.

TABLE VI.

*Summary of agglutination tests, showing number of strains agglutinated by different sera in various dilutions. The highest dilution at which each strain agglutinates is considered the dilution appropriate to that strain.*

	1/2000	1/500	1/100	1/20	1/5	None
<i>Immune Serum 1.</i>						
11 London strains	—	4	—	2	4	1
14 Epidemic „	—	—	—	3	4	7
<i>Immune Serum 2.</i>						
5 London strains	4	—	—	—	1	—
6 Epidemic „	1	—	1	2	2	—
<i>Immune Serum 3.</i>						
3 London strains	1	—	—	—	—	2
9 Epidemic „	—	2	1	3	—	3
<i>Normal Serum.</i>						
10 London strains	—	—	1	1	3	5
14 Epidemic „	—	—	—	1	3	10

Normal horse serum agglutinated one strain (Sporadic) in dilution of 1—100, two strains at 1—20, six at 1—5 and 17 not at all in dilution of 1—5.

Experiments on the power of four Sporadic and two Epidemic strains to absorb agglutinins from Serum 1, showed a power of absorption in the different strains which was to a certain extent proportional to their agglutinability.

Of the Sporadic strains two (L. 1 and L. 5) which were agglutinated about equally and in high dilution (1—500) absorbed their own and each others agglutinins, but absorbed to a much less extent the agglutinins of the other two Sporadic strains.

Strains L. 7 and L. 12 were not agglutinated so well as L. 1 and L. 5 by Serum 1. They were capable of absorbing to a certain extent their own and each other's agglutinins, but hardly removed any of those for L. 1 and L. 5.

The two Epidemic strains, which were only partially agglutinated in dilutions of 1—20 and 1—50, absorbed none of the agglutinins for themselves nor for the other strains.

This experiment then indicates a grouping of these six strains into three groups, viz. :

Group 1. Two Epidemic strains.

Group 2. Two Sporadic strains.

Group 3. Two Sporadic strains of intermediate character.

*The Meningococcus*

TABLE VII.

*Microscopic agglutination of six strains by Serum 1, before and after it has been absorbed by each strain. In each case 0·25 c.c. of serum after dilution 1/10 was used to emulsify one (24 hour) agar slope of the Meningococcus. The emulsion was incubated at 37° C. for two hours and the cocci removed by centrifugation.*

Strain of Meningococcus	Serum 1 absorbed by L. 1				Serum 1				Serum 1 absorbed by E. 7				Serum 1 absorbed by L. 7				Serum 1 absorbed by L. 12			
	1/20	1/50	1/100	1/200	1/500	1/20	1/50	1/100	1/200	1/500	1/20	1/50	1/100	1/200	1/500	1/20	1/50	1/100	1/200	1/500
L. 1	#	#	#	#	#	+	-	-	-	-	L. 1	#	#	#	#	#	#	#	#	#
L. 5	#	#	-	-	-	+	-	-	-	-	L. 5	#	#	#	#	#	#	#	#	#
E. 5	+	+	-	-	-	#	-	-	-	-	E. 5	+	-	-	-	+	-	-	-	-
E. 7	+	-	-	-	-	+	-	-	-	-	E. 7	-	-	-	-	-	-	-	-	-
L. 7	#	#	#	#	#	#	#	#	#	#	L. 7	#	#	#	#	#	#	#	#	#
L. 12	#	#	+	+	-	-	+	+	+	-	L. 12	#	#	#	#	#	#	#	#	#
Serum 1 absorbed by L. 5					Serum 1					Serum 1 absorbed by E. 5					Serum 1					
L. 1	#	#	#	#	#	-	-	-	-	L. 1	#	#	#	#	#	#	#	#	#	
L. 5	#	#	#	#	#	-	-	-	-	L. 5	#	#	#	#	#	#	#	#	#	
L. 7	#	#	#	-	-	#	#	#	-	L. 7	#	#	#	#	#	#	#	#	#	
L. 12	#	#	#	-	-	#	#	#	-	L. 12	#	#	#	#	#	#	#	#	#	
L. 1	#	#	#	#	#	+	#	#	#	L. 1	#	#	#	#	#	#	#	#	#	
L. 5	#	#	#	#	#	+	#	#	#	L. 5	#	#	#	#	#	#	#	#	#	
L. 7	#	#	#	+	+	#	#	#	+	L. 7	#	#	#	#	#	#	#	#	#	
L. 12	#	#	#	+	+	#	#	#	-	L. 12	#	#	#	#	#	#	#	#	#	

The Epidemic strains E. 5 and E. 7, which are not themselves agglutinated, absorb no agglutinins.

The agglutination experiments shew that numerous injections, extending over a long period, of two strains of Meningococci produced a serum which agglutinated in high dilutions the two homologous and a few other strains, but failed to agglutinate considerably more than half the strains tested.

The experiments also indicate an arrangement of the strains into groups. Each group would consist almost entirely of Epidemic or Sporadic strains. This is shown both by the agglutinations with the sera prepared at the Lister Institute and also by the use of Kolle's serum, assuming that this serum was prepared with strains all of which originated from Epidemic cases.

The variations in the agglutinability which occur among different strains of *Meningococcus* recorded here have a specific character and are not necessarily correlated with variations in their agglutinability by normal serum.

By the results of absorption experiments with Serum 1 the strains can be arranged into groups, the members of which are apparently closely related whilst having few if any agglutinins in common with the remainder, e.g., a group of the London strains containing L. 1, L. 5, L. 7, and L. 12 of which L. 1 and L. 5 form one sub-group and L. 7 and L. 12 another.

Amongst the Epidemic strains the results of agglutination and absorption closely connect E. 12 and E. 19 whilst shewing no relationship of these to the rest. The agglutination results do not enable us to differentiate accurately the Epidemic strains from the Sporadic strains since there are as great differences between the individual Epidemic and the individual Sporadic strains as between the members of the two groups. For instance, some Epidemic strains were agglutinated (1—25) by Serum 1 (Sporadic) while several Sporadic strains were not agglutinated at all.

The differences between various strains of Meningococci are then very great as regards agglutination. They are not, however, greater than those shown by Torrey (1907) to exist between different strains of Gonococci in respect of agglutination and also of power of fixing complement in the presence of an immune serum (Bordet's method).

#### FIXATION OF COMPLEMENT (BORDET'S METHOD).

A series of experiments made to test the power of Serum 1 (prepared with one Sporadic strain) to fix complement in a haemolytic system in

the presence of different strains of *Meningococcus* tended, on the whole, to confirm the differences and affinities between the strains as shewn by agglutination.

For these experiments on Fixation of Complement suspensions of agar cultures of *Meningococci* were used. A trial was made with extracts of the cocci, but the results did not appear to be so satisfactory, perhaps, because certain strains autolysed more readily than others.

The haemolytic serum used was the heated serum of a rabbit which had been immunised with ox corpuscles, and the red corpuscles were those of the ox, washed and suspended in a concentration which corresponded to 2 per cent. of the original blood.

The complement used was that of the guinea-pig diluted 1—10.

0·1 c.c. of the diluted complement was added to 0·3 c.c. of the emulsion of cocci and 0·3 c.c. of Serum 1 (from the horse injected with the Sporadic *Meningococcus* L. 1). This serum was used in varying dilutions, 1/1, 1/5, 1/25, 1/100 and 1/500.

The degree of correspondence between the particular strain of *Meningococcus* and the anti-*Meningococcic* serum was measured by the dilution of serum by which the complement was fixed as indicated by the absence of haemolysis, when the haemolytic serum and corpuscles were added.

The anti-*Meningococcic* serum in various dilutions, the cocci and the complement were mixed and put in the incubator at 37° C. for 40 minutes. To each tube were then added 1 c.c. of the emulsion of ox corpuscles and 0·1 c.c. of inactivated haemolytic serum diluted 1—10. The whole was then returned to the incubator and left at 37° C. for 1½ hours, after which the tubes were placed at the laboratory temperature for about 20 hours. At the final examination of the tubes it was found that Serum 1 in dilutions of 1—25 had completely prevented haemolysis with some strains of *Meningococci*, but with other strains the haemolysis had taken place as readily as when cocci or Serum 1 were absent.

The Sporadic *Meningococci* L. 1, L. 7, L. 12 and L. 8 prevented haemolysis completely or very nearly completely, while L. 5 prevented haemolysis in some experiments, but not in others. These were the only Sporadic strains used. On the contrary, five Epidemic strains did not prevent haemolysis.

A number of controls testing the powers of the individual constituents of the test tubes were made simultaneously and also a control made by substituting normal horse serum for Serum 1. These controls

in each instance gave a satisfactory result as shewn in Table IV. When other bacteria were used instead of Meningococci with Serum 1, either haemolysis took place as if no bacteria or serum were present or else the presence of these bacteria prevented haemolysis even in the absence of serum. The bacteria used as controls in this way were *Staphylococcus*, *Micrococcus catarrhalis* and *B. diphtheriae*.

## SUMMARY OF FIXATION OF COMPLEMENT EXPERIMENTS.

Of five Sporadic strains of Meningococcus tested with Serum 1 (prepared with one Sporadic strain) one (homologous) strain prevented haemolysis when the serum was diluted to 1-100, three strains when it

TABLE VIII.

*Fixation of Complement in a Haemolytic System by different strains  
of Meningococci in presence of Serum 1.*

Complement = Fresh guinea-pig's serum.

H. S. = Rabbit's serum (heated) Immune v. Ox corpuscles.

# = Complete haemolysis.

# = Marked haemolysis.

= No haemolysis

± = Slight haemolysis.

\* Normal serum used instead of Serum 1 in these tubes.

The dilution 1-25 is the series which best shews difference of strains.

The dilution 1-25 is the serial which best shows difference of strains.

The Serum 1 of horse immunised with one Sporadic strain (L. 1) prevents haemolysis in presence of an emulsion of Strains L. 1, L. 12, L. 8, L. 7 (all Sporadic strains), but not in the presence of E. 2, E. 4, E. 7, and E. 12 (Epidemic strains).

was diluted to 1—25 and one strain gave a variable reaction. Of five Epidemic strains used none did more than partially prevent haemolysis when the dilution was 1—25.

TABLE IX.

*Summary of Fixation of Complement Experiments.*

	Dilution of undiluted Serum c.c.	Amount of Serum c.c.	Emulsions of Meningococcus strains 0·3 c.c. of each								
			L. 1	L. 5	L. 7	L. 8	L. 12	E. 2	E. 4	E. 5	E. 7
											E. 12
Anti-Meningococcic	1/5	.06	—	±	—	—	—	#	#	+	+
Serum 1	1/25	.012	—	±	—	—	—	##	##	##	#+
	1/100	.003	—	#	+	#	+	##	##	##	#+
Normal Serum	1/1	.3	##	##	##	##	##	#	##	##	##

# = Complete haemolysis.

= Marked haemolysis.

+ = Slight haemolysis.

— = No haemolysis; complement completely fixed.

## IMPORTANCE OF THE DIFFERENCE BETWEEN STRAINS.

In assessing the value to be put on the differences in sugar fermentation and serum reactions between different strains of Meningococcus, as indications for division of the group into sub-species, the similar variations occurring amongst other groups of bacteria and especially amongst the members of the closely allied species of Gonococcus must be borne in mind.

As mentioned above, Dunn and Gordon (1905) state that the Gonococcus ferments Glucose but not Maltose, whereas Wollstein (1907) and Gurd (1908) found that Glucose and Maltose were both fermented by a large number of strains of Gonococcus. Of two strains of Gonococcus which I isolated from different cases of acute urethritis in male patients the one produced acid from Glucose and Maltose, the other from Glucose but not from Maltose. The sugar reactions of the Gonococcus are therefore not constant.

As regards agglutination, fixation of complement and the formation of antibodies, great similarity has been shown to exist between some strains of Meningococcus and some strains of Gonococcus by Bruckner and Cristéanu (1906), Wollstein (1907), Teague and Torrey (1907), Gurd (1908), and Dopter and Koch (1908).

The differences between the individual races of Gonococcus, or of Gonococcus as tested by these methods, appear to be almost as great as between the two species.

It is, therefore, unlikely that well-marked constant differences along these lines will be found between the groups and sub-divisions of the whole class to which the Gonococcus and Meningococcus both belong.

If specific differences between the two groups of Meningococci exist, other methods must be found than those detailed above by which to demonstrate them. Of the Opsonic test as described by Houston and Rankin (1907) and also used by Taylor (1907) I have no experience.

One feature of the group of Meningococci from Sporadic cases taken as a whole appears to be that its members are more frequently found to deviate from the type to which most strains conform, than is the case with the Epidemic group.

The great variability among the strains shown above points strongly to the desirability of employing a polyvalent serum for therapeutic purposes.

#### ABERRANT STRAINS OF THE MENINGOCOCCUS, AND ORGANISMS LIABLE TO BE CONFUSED WITH THE MENINGOCOCCUS.

Apart from its morphology the Meningococcus possesses certain characteristics which are almost invariable, viz. (1) its inability to grow at 24° C. and (2) the property which allows a young agar culture to be easily made into a uniform suspension which does not shew spontaneous agglutination.

The other characters are not quite constant and some strains occur which deviate further than usual from the accepted type of the species. For instance, strains L. 13, E. 3 (and E. 4 in peptone water) did not ferment any of the sugars used, though they resembled typical Meningococci in every other way and E. 3 and E. 4 were agglutinated by Kolle's anti-Meningococcic serum.

Another strain L. 5 at one period of its culture fermented no sugars though at a later period it produced acid from Glucose and Maltose. L. 3, obtained in pure culture during life from the cerebro-spinal fluid of a case of posterior basic meningitis, gave the usual sugar reactions and agreed with typical Meningococci in every way except that it grew very slowly at 24° C. This strain was mentioned by me in a previous paper (Arkwright, 1907).

Strain L. 9 (2) may be mentioned here though it is not implied that

it was a Meningococcus. This strain closely resembled *Micrococcus catarrhalis*. It grew at 20° C., fermented no sugars, resisted attempts to make a uniform suspension of the cocci, clumped spontaneously, and was of characteristic appearance in cultures and microscopically. It was obtained post-mortem from the heart-blood of a case of Sporadic meningitis from the cerebro-spinal fluid of which during life a typical Meningococcus (L. 9 (1)) was isolated.

It remains to mention some interesting micro-organisms which were, perhaps, all of the same species and which were isolated from the cerebro-spinal fluid of three cases of Sporadic meningitis.

These organisms, in stained films, from the cerebro-spinal fluid or from young agar cultures appear as gram-negative cocci which might well be taken for Meningococci. Two of these strains were isolated by Dr W. E. Marshall, to whom I am indebted for them, and one strain was isolated by myself. These strains are denoted in the tables as L. 6, L. 15 and L. 23. One of them (L. 6) was recorded in a former paper as a Gram-negative coccus resembling the description of *M. cinereus* given by v. Lingelsheim (Arkwright, 1907). I think, however, that it probably belongs to the present group. The characters of L. 15 and L. 23 have been more particularly examined and present the following features:— a growth on agar or gelatine at 22° C. is quite distinct in 24 hours; it is transparent and soon becomes confluent. The colonies appear bluish grey by reflected light and yellowish by transmitted light. Glucose, Maltose, Laevulose, and Cane sugar are not fermented. In broth the growth causes uniform turbidity. On MacConkey's bile salt agar there is fair growth. Uniform suspensions of an agar culture in salt solution are easily made and in the case of two strains no spontaneous clumping occurred, but in a suspension of the third strain some clumping occurred without the addition of serum.

Morphology:—A film made from a 24-hours' agar culture and stained by Gram's method with neutral red as a counterstain shews coccal forms in coherent masses or in short lines of 4 or 5. These cocci are rather smaller than most Meningococci, are of uniform size and mostly round, sometimes with an unstained dividing line across the middle. Some double forms like typical Meningococci are present but not in great numbers. In some fields, one or two long bacillary forms are seen which stain rather more deeply with the neutral red than the cocci, and which often shew indications of division into three or four segments. These bacilli are always present though sometimes in very small numbers and are more frequent in older cultures.

In broth cultures the morphology is more that of a short oval bacillus with some long forms.

At first the bacillary forms were regarded as due to a contamination, but microscopical preparations from isolated colonies after plating always presented the same appearance.

Similar organisms were isolated from cases of meningitis by W. James Wilson (1908).

As to the pathogenicity of these Gram-negative organisms and of others more closely resembling *M. catarrhalis*, which have been isolated from the cerebro-spinal fluid or blood in cases of meningitis it would be rash to give too positive an opinion at present. They apparently have no relationship to the Meningococcus of Weichselbaum. They may however be the causal organism in some cases of meningitis, or may play a secondary part.

#### CONCLUSIONS.

1. Whilst most Meningococci produce acid from Glucose and Maltose, strains of undoubted Meningococci are met with which at some stage of their artificial culture ferment only one or neither of these sugars.

2. Other minor differences in the fermentation reactions also occur amongst undoubted Meningococci.

3. These atypical varieties are found amongst strains from both Epidemic and Sporadic cases of meningitis, but more frequently come from Sporadic cases.

4. Specific agglutination in high dilutions (1—500 to 1—2000) obtained with the serum of a horse injected with two strains of Meningococcus was limited to a very few strains besides those used for the inoculation.

5. Agglutination experiments after the absorption of agglutinins tended to further mark the division of the strains of the Meningococcus used into sub-groups.

6. Experiments on the fixation of complement by the specific serum in the presence of different strains of Meningococcus confirmed on the whole the grouping indicated by the agglutination experiments.

7. The variations observed in the sugar and serum reactions were not such as to indicate a specific difference between the Epidemic and Sporadic strains, for the differences between individual members of each group were as great as any found between the two groups.

8. A Gram-negative coccus resembling more nearly the *Micrococcus catarrhalis* than the Meningococcus was found in the blood post-mortem of a case of Meningococcal meningitis.

9. A Gram-negative bacillus, whose morphology was chiefly that of a micrococcus and closely resembled that of the Meningococcus in the cerebro-spinal fluid and in young cultures, was found in pure or almost pure culture, in the cerebro-spinal fluid of three cases of Sporadic meningitis.

I have very much pleasure in recording my thanks to Dr George Dean for the help which he has given me throughout the research, and to Dr A. T. MacConkey for carrying out the work connected with the immunisation of a horse at Elstree.

#### REFERENCES.

- ANDREWES, F. W. and HORDER, T. J. (1906). A Study of the Streptococci pathogenic for man. *Lancet*, Vol. II. p. 775.
- ARKWRIGHT, J. A. (1907). On Variations of the Meningococcus and its differentiation from other cocci occurring in the Cerebro-spinal fluid. *Journ. of Hyg.* Vol. VII. p. 193.
- BRUCKNER and CRISTÉANU (1906). Sur les précipitines du Gonocoque et du Méningocoque. *Compt. rend. d. Soc. de Biol.* 1906, Vol. I. p. 1070.
- DOPTER, CH. and KOCH, RAYMOND (VII. 1908). Sur la coagglutination du Méningocoque et du Gonocoque. *Compt. rend. d. Soc. de Biol.* Vol. II. p. 215.
- (x. 1908). Sur les précipitines du Méningocoque et du Gonocoque. *Compt. rend. d. Soc. de Biol.* Vol. II. p. 285.
- DUNN, R. A. and GORDON, M. H. (1905). On the Clinical and Bacteriological aspects of an Epidemic simulating Influenza. *Brit. Med. Journ.* Vol. II. p. 421.
- GURD, F. B. (1908). A contribution to the Bacteriology of the female genital tract with special reference to the detection of the Gonococcus. *Journ. of Med. Research*, Vol. xviii. p. 291.
- HOUSTON, T. and RANKIN, J. C. (1907). The Opsonic and Agglutinative action of blood serum in Cerebro-spinal fever. *Brit. Med. Journ.* Vol. II. p. 1414.
- v. LINGELSHHEIM, W. (1906). Die bakteriologischen Arbeiten der Königl. Hygienischen Station im Beuthen in O. Schl. während der Genickstarre Epidemie im Winter 1904—5. *Klin. Jahrb.* Vol. xv. p. 373.
- TAYLOR, A. (1907). The Opsonic Index and Agglutination in cerebro-spinal meningitis. *Lancet*, Vol. II. p. 16.
- TEAGUE, O. and TORREY, J. C. (1907). A Study of Gonococcus by the Method of "Fixation of Complement." *Journ. Med. Research*, Vol. xvii. p. 223.
- TORREY, J. C. (1907). Agglutinins and Precipitins in Anti-Gonococcic serum. *Journ. Med. Research*, Vol. xvi. p. 329.

- TRAUTMANN, H. and FROMME, W. (1908). Beiträge zur Epidemiologie und Bakteriologie der epidemischen Genickstarre. *München. med. Wochenschr.* p. 791.
- TWORT, F. W. (1907). The Fermentation of Glucosides by Bacteria of the Typhoid-colic group and the acquisition of new fermenting powers by *Bacillus dysenteriae* and other micro-organisms. *Proc. Roy. Soc. B.* Vol. LXXIX. p. 329.
- WILSON, W. J. (1908). A contribution to the bacteriology of cerebro-spinal meningitis. *Lancet*, Vol. I. p. 1686.
- WOLLSTEIN, M. (1907). Biological relationships of *Diplococcus intracellularis* and *Gonococcus*. *Journ. Exper. Med.* Vol. xi. p. 588.

## A NOTE ON EXPERIMENTAL LEAD POISONING.

By KENNETH GOADBY, D.P.H. (CAMB.).

*(From the Laboratory of Pathological Chemistry, University College Hospital, London.)*

THE incidence of Industrial Lead Poisoning is closely related to those sections of the manufactures which involve the production of dust. In white-lead works, paint-grinding shops, litharge grinding, electrical accumulator works, etc., the dust takes the form of minute particles of the special compound of lead manipulated by the workman. In smelting, desilverising, trimming metals and in file cutting, finely divided metallic lead, or a lead oxide, is present in the workshop air.

In a Special Report on Lead Poisoning in Paint and Colour Manufactures, Dr T. M. Legge points out that at least 80 per cent. of the cases of poisoning collected by him in paint workshops are related to the processes in which the lead compound is handled in the dry state (dusty processes).

It is also commonly recognised that the special danger in emptying Dutch stoves in the white-lead works, and mash-making in paint-grinding works, to take two specific instances, comes from the white-lead dust thrown into the air; and further that, when proper provision is made for the withdrawal of the dust by powerful exhaust fans, the amount of poisoning associated with these two processes is greatly diminished. The substitution of one process for another is also often followed by a distinct diminution in the incidence of the poisoning, and I have been able to show that the decrease in the poisoning in a white-lead factory followed the introduction of a particular change in the method of setting the beds, so that the resulting corroded lead was much harder and therefore freer from dust than in the original process,

and therefore less dust was produced in its removal when the stacks were emptied.

From the undoubtedly association of lead poisoning with the dusty operations in the manufacture of lead compounds, I thought it of some interest to enquire experimentally into lead poisoning by subjecting animals to the inhalation of lead dust in air. The best animal to use for such experiments was at once suggested by the fact that cats and dogs when allowed to roam at will in a white-lead works rapidly become poisoned, and ultimately die with similar symptoms to those observed in man. "It is impossible to keep a cat, they always die of lead poisoning here," is a common saying in the lead factories. Rats, with which many of the factories swarm, appear to be immune, and are never found dead or dying from lead poisoning. Cats were used for this reason, and because they are easy to deal with, and such animals as guinea-pigs are somewhat too small, and, in common with rabbits, differ more in their alimentary system from man than do cats. A further series of experiments, to be published later, have also been carried on with cats, alcohol being given in addition to the lead, and causing a more rapid appearance of the symptoms of lead poisoning than when lead alone is used.

The lead dust in air may presumably be absorbed through two channels:

(a) The gastro-intestinal canal—by the dust in the air becoming deposited upon the pharynx and nasal mucous membrane and then swallowed.

(b) By direct inhalation of the particles of dust into the lung.

That dust can gain direct access to the lungs is evidenced by the dark stained sputum in persons exposed to dusty atmospheres, as coal miners, grinders, etc.; and the following experiment shows the difficulty of removing white-lead dust from the air by moist chambers or water.

Four 750 c.c. wash bottles were arranged in series with india-rubber tubes so that air could be drawn through the apparatus by means of a water pump. The whole apparatus was washed out with water, including the rubber junction tubes  $\frac{1}{4}$  inch in diameter, and some 2 ft. long. A bottle containing white-lead dust was attached to the distal end and gently shaken in the hand. Within three minutes of the pump being started white-lead dust was observed in the proximal flask. The rate of flow was two litres in 35 secs. The dust had passed through three sets of wet  $\frac{1}{4}$  inch rubber tube and through the three moist wash bottles.

There therefore appears to be considerable probability that white-

lead dust can find its way into the lung direct, and as in the white-lead and paint-making industries the workmen are frequently subjected to sudden puffs of white-lead dust an attempt was made to reproduce in an experimental manner the working conditions. It was necessary however to somewhat shorten the period during which the experimental animals were exposed to inhalation of lead dust, so somewhat larger quantities of lead dust were used over shorter periods than would be the actual case in a paint mill or other industrial process. This point will be referred to again.

#### *Apparatus.*

For the purpose of the experiments a large cage was constructed 6 ft. by 3 ft. 6 ins. by 3 ft., having the two long sides and the top glazed so that the animals could be watched during the whole of the experiment; the remainder of the cage was lined with sheet zinc. At one end was placed an electric fan making 2000 revolutions per minute. Immediately in front of the fan and in the roof of the cage was a  $\frac{3}{4}$  inch hole through which passed the stem of an ordinary glass funnel. This was covered with a piece of sheet lead through which passed a rod terminating inside the cage in a disc so arranged that it could be pulled up (without opening the cover of the funnel) and so prevent the white-lead or other lead compound from falling through the funnel until required. At the end of the cage farthest from the fan a wire cage was placed in which the animal could lie down without discomfort. Through the back wall on a level with the animal's head a 1 inch hole was drilled through the cage for the purpose of taking air samples. A cork, through which passed a glass tube plugged with cotton wool, was pushed into the hole. The tube was then connected with an aspiratory jar and a measured quantity of air drawn through the cotton wool plug, in which the lead present was afterwards estimated. Two large holes plugged with cotton wool were also made in two other situations to allow of ventilation in the cage. A fourth hole was made through the side of the cage near the fan, through which a glass tube could be passed and steam let into the cage at the termination of the experiment. Two doors were also provided for manipulating, cleaning, etc.

#### *Method of Experiment.*

The cage was in the first place well cleaned out after the previous experiment by means of steam and then water, followed by mopping

out and drying by a small charcoal stove and hot bricks. It was then well ventilated by running the fan with both doors open. The animal under experiment was then placed in the cage and the doors closed tightly. A weighed quantity of the lead compound well dried and in fine powder was then placed in the funnel and the fan started. Small quantities of the dust were now allowed to drop into the cage by lowering or raising the rod through the funnel; as the dust dropped some was taken up by the motion of the fan and stirred up into the cage air. The dust was allowed to fall gradually, about ten minutes being required to pass in 50 grms. The respirations of the animal were noted during the experiment. At the end of 15 minutes a further quantity of dust was started in the manner described above, and at the end of 30 minutes the fan was stopped. Steam was then turned into the cage for a few minutes, after which the door was quickly opened and the animal removed, taken outside the laboratory and thoroughly brushed with a hard brush to remove all dust adhering to its coat.

Immediately the first quantum of dust had been added to the cage air five litres of air were aspirated off in about 60 seconds through the glass tube plugged with cotton wool.

If the animal showed any disposition to lick itself during the experiment it was immediately stopped, but as a rule the cats curled up and went to sleep either facing, or with their backs to the fan; they made no attempts to get out of the wire cage and appeared entirely indifferent to their surroundings. In only a few instances did they cough or sneeze, white-lead dust in particular being evidently non-irritating, whereas litharge dust on several occasions caused coughing. I have noticed a similar difference between the dust produced in litharge and white-lead grinding in a factory. The litharge dust is much heavier than the white lead and becomes deposited on the cage walls more rapidly. Flue dust on the other hand is very fine and circulates longer in the cage air.

#### *Lead compounds used.*

Three varieties of lead compound were used in these experiments: three animals (cats) were experimented with, a fourth cat being used as control and fed with a lead compound, and a fifth kept in the laboratory as a general control.

The compounds of lead used were:—

A. *Flue dust* from the "market plot" in desilverising. This dust

contains from 50 % to 60 % PbO. The dust is produced as follows. In the process of desilverising zinc is added to the molten lead containing the silver and gold, and the zinc, which contains practically all the precious metals, is separated from the lead by the difference of melting point, etc. The "poor lead," or lead from which the zinc has exhausted the precious metals, is run into a pot called the "poor pot" or "market pot," holding some 30 tons of metal. Air and steam under pressure are then forced through the molten mass, oxidising the zinc and at the same time some of the lead. The exhaust from the pot passes into a flue and underground chamber, where the dust is collected. A considerable amount of dust finds its way into the air of the factory during the process of blowing.

B. *Litharge* obtained from the air ducts leading from a litharge grinding plant. This dust may escape and be inhaled by the persons engaged on the grinding machine. The litharge lumps are broken up by hand and shovelled into the hopper of the grinding machine.

C. *White lead* obtained from the air ducts leading from the packing machines used in packing dry white lead into barrels. Persons engaged in white-lead or paint manufacture are liable to inhale this dust. White lead from the air ducts of the packing machines etc. finds its way to the potteries as "off colour lead" and is used in making glazes and fritts.

#### EXPERIMENTS<sup>1</sup>.

A. *Flue dust*. Cat w. = 3 kgs. subjected to inhalation of "flue dust" on 10 occasions from Nov. 15th to Dec. 18th, 1905. Final weight 2·5 kgs., loss = 0·5 kg.

Colic Dec. 3rd and Dec. 18th. Extensor paralysis of front paws, weakness of back muscles, slight retinal haemorrhage.

B. *Litharge*. Cat w. = 3·77 kgs. subjected to inhalation of litharge dust on 10 occasions, Nov. 22nd to Dec. 18th, 1905. Final weight 3·0 kgs., loss = 0·77 kg.

Slight paralysis of hind limbs, no eye changes. Colic Dec. 6th.

C. *White lead*. Cat w. = 4·1 kgs. subjected to inhalation of white lead on eight occasions, Nov. 28th to Dec. 18th, 1905. Final weight 3·04 kgs., loss = 1·06 kg.

Dec. 1st, Colic, hind leg stiff and extensor paralysis. No eye changes.

<sup>1</sup> Full details of the experiments are given in the Tables.

D. *Flue dust Control.* Cat w.= 2·75 kgs. Fed with flue dust.

Nov. 16th, 1905 to Dec. 18th, 1905, Sundays excepted.

Total quantity taken 7·25 grms.

Slight stiffness hind limbs, no retinal change, Colic Dec. 7th.

The method first tried for estimating the amount of lead present in the cage air was as follows. The cotton wool in the glass tube was dissolved in dilute nitric acid and the contained lead precipitated and weighed as sulphate.

This method was found to give somewhat unequal results, and was discarded, the lead being dissolved out of the cotton wool with nitric acid, filtered, and finally determined by the molybdate method with tannic acid as indicator.

It will be seen that the amount of dust used was gradually increased, 20 grms. and finally two lots of 30 grms. being used during the experiments. It will also be noticed that a considerable variation in the respirations occurred, particularly with the cat which was subjected to the litharge dust. I noticed throughout that the litharge dust was apparently much more irritating to the lung, and produced far more discomfort than the other two varieties of dust used. Both the white-lead cat and flue-dust cat remained for the most part quite quiet during the experiments, the cats going to sleep during the whole of the time; but with the litharge cat the animal very rarely remained still, was constantly sneezing, and was apparently feeling distinctly uncomfortable. It never sat down and went to sleep like the other cats, whilst its respirations were more frequent than the flue-dust or white-lead cats.

It will be noticed on comparing the weights of the cats that the flue-dust cat shows the same amount of diminution in weight as the cat fed on the flue dust. All the cats show progressive diminution in their weight. Control cats kept under similar conditions in the laboratory increased considerably in weight when not fed with lead dust, and it was noticed in the experimental cats that the diminution in weight was rather rapid at first.

The first noticeable change in the experimental cats was an alteration in the appearance of the face due to the wasting of the orbital and buccinator fat giving the animal a pinched appearance curiously like the facial appearance presented by men who have worked for long periods in dangerous positions in lead works.

Colic was the next symptom observed, the cats first showing a disinclination to eat their food, and then showing signs of abdominal discomfort, which at times became more pronounced. Obstinate con-

stipation appeared about the same time, the faeces of the experimental cats contrasting sharply with those of the control cats in the laboratory fed on the same diet without exposure to lead.

Weakness of the muscles was also noticed, especially the muscles of the back and the extensor muscles of the limbs, the latter causing the animal to adopt a curious stiff gait. It walked on the tips of its toes, and in endeavouring to turn round was obliged to arch its back and draw its feet close together to prevent its falling over. When attempting to turn when running down the laboratory after a ball it invariably fell over. The back muscles were distinctly weak, so much so that if the animal was held up by placing one's forefinger and thumb behind its ears it hung straight, and any attempt to twist or claw the holder's hand was impossible, whereas a normal cat held in the same way soon causes itself to be dropped. When jumping off a table on to the floor, the cat fell upon its belly, the extensor muscles being evidently unequal to the strain.

The four cats were further examined with an ophthalmoscope for eye changes, but in only one, the flue-dust cat, was any distinct eye change noticed, and the examinations had to be discontinued as the use of the atropine even in small doses caused acute salivation and running from the nose.

The loss of weight appeared at first progressive, but when the loss had proceeded to a certain point the weight remained fairly constant, the white-lead cat alone showing a tendency to rise in weight at the termination of the experiments. Glibert, who fed rabbits with pills of white lead, found there was no great loss of weight and that the loss apparently had no relation to the onset of symptoms of acute poisoning. My experiments were not carried as far as the death of the animal by acute poisoning, all the animals being killed when distinct signs of poisoning had supervened.

No blood examinations were made, as the examination of persons working in lead factories, although showing a certain degree of secondary anaemia, does not show any specific changes.

A few experiments were made in addition to the inhalation observations, regarding the solubility of the white lead and litharge in gastric juice obtained from a normal man.

The two samples<sup>1</sup> of gastric juice were obtained by means of a stomach tube after a test breakfast of tea and toast taken fasting.

<sup>1</sup> Both samples were tested for digestive efficiency and acidity. No. 2 shewed a slight excess of both total and volatile acidity over normal gastric contents.

To 10 c.c. of each of these samples of gastric juice were added:

- |                           |   |
|---------------------------|---|
| A. 0·1 grm. Lead Sulphate | } |
| B. 0·1 " White Lead.      |   |
| C. 0·1 " Litharge.        |   |

The mixtures were then digested at 37° C. for one hour: the digests centrifuged; and 2 c.c. of the supernatant fluid removed with a pipette and titrated against an ammonium molybdate solution, 1 c.c. of which was equal to 0·0008 grm. PbO. The digests were made in duplicate and two estimations made of each.

The average quantities of PbO present in the digests were:

- |                               |   |
|-------------------------------|---|
| 1. A. Lead Sulphate. 0·080 %. | } |
| B. White Lead. 0·048 %.       |   |
| C. Litharge. 0·040 %.         |   |
| 2. A. Lead Sulphate. 0·046 %. | } |
| B. White Lead. 0·042 %.       |   |
| C. Litharge. 0·034 %.         |   |

It would appear therefore that very little difference exists in the solubility of the three compounds in normal gastric juice, but that, if anything, the lead sulphate is the more soluble. The observation tends to throw doubt on the advantage of the sulphuric acid sanitary drink in use in the various lead works.

Certain objections suggest themselves in the foregoing inhalation experiments. Firstly it may be objected that the animals would swallow the lead dust in the air breathed, the dust becoming deposited upon the pharynx and nasal mucous membrane and so swallowed. There is, however, sufficient evidence in both the wash bottle experiment and in the production of industrial diseases in other trades than lead works, that dust finds its way into the lung. Miners' phthisis, grinders' rot, stone masons' phthisis, are cases in point, while in the particular case of the experiments detailed the quantity of lead present in the cage air was so small that the animal if it swallowed the whole of the dust it breathed in would not obtain as much as the control cat which was fed with lead.

The further criticism that the animals obtained lead dust from their coats is not, I think, a serious one, for the greatest care was exercised after each experiment to thoroughly brush and dust the animal's coat before it was returned to the cage.

A further objection that arises is the fact that the animals were subjected to somewhat more severe conditions than those under which industrial lead poisoning takes place. The objection is not so serious as would at first sight appear. A man engaged in tipping barrels of dry white lead into a hopper of a paint-grinding mill, unless the mill hopper be provided with a very efficient exhaust draught, gets a puff of dust into his face at the moment of emptying the white-lead barrel, a process that is continued at intervals during the day. Again, in the lower part of the paint mill at the moment of mash-making dust generally finds its way out at the lower levels. In stripping white-lead beds during the emptying of the earthenware pots into the trays clouds of dust are frequently produced, and when the corosions are placed in the washing apparatus or wash beck a cloud of dust is always given off if the workman is in a hurry or careless. Although therefore the workman is not working in a confined space and having clouds of lead dust blown into his face for half an hour, as were the experimental animals, yet during the day and week he is subject to constant small doses of lead dust; and, if at any time the dose inhaled is somewhat larger than his metabolic process can easily deal with, some evidences of poisoning supervene. So much is this the case that a break-down of the dust preventing apparatus in a white-lead factory is generally followed in a day or two by symptoms among the men working, while the poisoning is always more associated with the dusty parts of the machinery or processes than the actual handling of the raw material.

Further, the men employed in the dusty processes are frequently covered with lead dust, and it is no uncommon thing to be able to wipe off distinct white-lead dust from a workman's eyelids after he has performed certain manipulations. Even should the amount of lead dust inhaled by the animals much exceed that of industrial processes, poisoning undoubtedly does take place in a dusty lead process, and the fact that the animal becomes poisoned somewhat more rapidly does not militate greatly against the cause of the poisoning. The largest number of cases of poisoning by lead reported from white-lead and paint-grinding works are first attacks occurring during the first nine months of exposure.

The experiments detailed above were performed for an essay submitted to the International Labour Bureau in 1906. Since that date the experiments have been considerably extended, my friend Dr F. W. Goodbody having collaborated with me, and we hope shortly to publish

our results, which are entirely confirmatory of the experiments detailed herein.

My experiments on the inhalation of lead dust show that poisoning by lead may take place through the lung as well as through the gastro-intestinal tract, and that susceptible animals exposed for short periods to air laden with lead dust develop symptoms of lead intoxication in a way analogous to the poisoning which takes place in men engaged in industrial processes, and exposed to the deleterious effects of lead-dust laden air.

Finally, as confirmatory evidence, it is interesting to note that at the end of the first fortnight of the experiments both myself, my friend, and the laboratory assistant all developed colic and constipation simultaneously during the week-end. On examining the cage we found a number of cracks where lead dust had undoubtedly escaped into the laboratory air at a point on a level with our faces; after these were properly closed no further trouble was experienced.

TABLE I.

*Lead Dust Inhalation Experiments.*

Animal No. 1. Cat. Weight = 3·000 kgs. Market Pot dust (PbO 50 %, ZnO 50 %). Details of the exposures in the special cage.

Date	Weight of animal kgs.	Total quantity of dust used gms.	Duration of exposure mins.	Average respirations per minute	PbO gms. per litre cage air	Notes
Nov. 15. 05	3·000	12	30	24	0·0019	
,, 17. 05	3·300	12	60	34	0·003	
,, 22. 05	2·800	40	60	40	0·003	
,, 28. 05	2·650	40	60	57	0·003	
Dec. 1. 05	2·500	40	20	45	0·004	Face pinched. Vomiting.
,, 3. 05	2·500	60	11	70	0·0014	Colic.
,, 6. 05	2·500	60	20	30	0·0016	Slight extensor paralysis.
,, 8. 05	2·500	60	20	35	0·0020	Back muscles weak.
,, 13. 05	2·750	60	15	30	0·0014	Colic.
,, 18. 05	2·250	60	20	20	0·0017	? Retinal haemorrhage.

TABLE II.

*Lead Dust Inhalation Experiments.*

Cat No. 3. Litharge dust from packing and grinding machine. (Cyclone dust collector.) Animal exposed to dust as No. 1.

Date	Weight of animal kgs.	Total quantity of litharge dust used gms.	Duration of experiment mins.	Average respirations of animal per min.	PbO gms. per litre in cage air	Notes
Nov. 22. 05	3.770	20	30	91	0.00019	
,, 24. 05	3.770	40	60	103	0.0001	Jerky respiration.
,, 28. 05	3.590	40	60	125	0.0558	Animal sneezing and coughing.
,, 29. 05	3.250	40	20	68	0.0781	Do. do.
Dec. 3. 05	3.300	40	20	87	0.0031	
,, 6. 05	3.300	60	20	81	0.0033	Animal fell over. Slight paralysis of hind limbs.
,, 8. 05	3.000	60	30	79	0.0026	
,, 11. 05	3.000	60	26	58	Lost	
,, 13. 05	3.000	60	20	33	0.0020	
,, 18. 05	3.000	60	20	65	0.0016	Colic. No eye changes.

TABLE III.

*Lead Dust Inhalation Experiments.*

Cat No. 4. White-lead dust from air duct of packing machine. Animal exposed as No. 1.

Date	Weight of animal kgs.	Total quantity of white-lead dust used gms.	Duration of experiment mins.	Average respirations per min.	PbO gms. per litre in cage air	Notes
Nov. 28. 05	4.00	40	30	43	0.0033	
,, 29. 05	4.300	40	20	44	0.0043	
Dec. 1. 05	4.000	60	20	40	0.00502	Colic.
,, 3. 05	3.500	60	20	26		Asleep during exp.
,, 6. 05	3.500	60	20	33	0.00273	Colic.
,, 8. 05	3.500	60	30	23	0.00273	
,, 11. 05	3.000	60	20	26	0.00262	
,, 13. 05	3.000	60	20	31	0.00273	Hind limb stiff. Colic.
,, 18. 05	3.040	60	20	26	0.00192	No eye changes.

TABLE IV.

*Control Experiment, Cat fed with Flue dust.*

Dust mixed with food (fish), given in first lot of food in the day.

Date	Weight of animal, kgs.	Quantity of PbO taken in dust, gm.	Notes
Nov. 16. 05	2·750	0·05	
.. 17. 05	2·750	0·05	
.. 18. 05	—	0·05	
.. 20. 05	2·500	0·10	
.. 25. 05	—	0·10	
.. 26. 05	—	—	
.. 27. 05	—	0·10	
.. 28. 05	—	0·10	Vomiting.
.. 29. 05	—	0·10	
.. 30. 05	—	0·10	
Dec. 1. 05	—	0·10	
.. 2. 05	—	0·10	
.. 3. 05	—	—	
.. 4. 05	2·000	0·5	Dose increased, as no symptoms.
.. 5. 05	—	0·5	
.. 6. 05	—	0·5	
.. 7. 05	—	0·5	Colic.
.. 8. 05	2·000	0·5	
.. 9. 05	—	0·5	
.. 10. 05	—	—	
.. 11. 05	—	0·5	
.. 12. 05	—	0·5	
.. 13. 05	—	0·5	
.. 14. 05	—	0·5	
.. 15. 05	—	0·5	
.. 16. 05	—	0·5	
.. 17. 05	—	—	
.. 18. 05	2·000	0·5	Slight stiffness in hind limbs, retinal arteries tortuous?

## PUBLICATIONS RECEIVED.

## BOOKS.

BOYCOTT, G. W. M. (1909). *Compressed Air Work and Diving*. London: Crosby, Lockwood & Son, 7, Stationers' Hall Court, Ludgate Hill. 116 pp., 16 figs., 25 x 16 cm. Cloth.

The book presents the main principles of compressed air work and diving, emphasis being laid upon the very important work which has of late been carried out by the Admiralty Committee on Deep Water Diving and by Drs J. S. Haldane and A. E. Boycott and Lieutenant Damant, R.N. (see this *Journal*, Vol. VIII.), which has led to altered regulations relating to diving and compressed air work. The author gives the rules for stage decompression and presents a good deal of new material to the reader. Mr Boycott, who is an Engineer, is to be congratulated upon his book, which promises to be of great use as a practical guide. The book is well printed and well illustrated.

BROOKE, G. E. (1909). *Essentials of Sanitary Science*. London: Henry Kimpton, 13, Furnival Street, Holborn, E.C. 413 pp., 62 figs., 19 x 13 cm. Cloth. Price 6/- net.

This book is intended as a manual for D.P.H. Students and "aims at covering all the necessary ground" required at examinations for the Diploma. The author has compiled the book from various sources, including his "own D.P.H. notes (compiled during reading and laboratory work)." The book certainly contains a great deal of information condensed into a small space.

DÜRCK, H. (1908). *Untersuchungen über die pathologische Anatomie der Beri-beri. Ein Beitrag zur normalen und pathologischen Anatomie des peripherischen Nervensystems*. Jena: Verlag von Gustav Fischer. 176 pp., 41 plates, 25 x 17 cm. Cloth.

Prof. Dürck's investigations, which are published in this book, were undertaken on behalf of the Deli-Maatschappij in Amsterdam. The author collected his beri-beri material in Sumatra, the Federated Malay States, Singapore, etc., and with the assistance of the artist, Karl Dirr, and first class lithographers has brought forth a work which, in the matter of beauty of illustrations, appears almost unique. Owing to the generosity of the Deli-Maatschappij no expense has been spared in the production of this volume, which represents work of the first order.

FOWLER, J. S. (1909). *Infant Feeding*, a practical guide to the artificial feeding of infants. London: Henry Frowde, Oxford University Press. 230 pp., 23 figs., 12 charts, 18 x 12 cm. Price 5/- net. Cloth.

This book, which forms one of the "Oxford Medical Publications" series, is based on lectures delivered by the author in Edinburgh to medical students and

post-graduates. As stated in the preface, the author has been content, for the most part, to speak of methods which he has personally tried and found useful.

GRESSWELL, G., and GRESSWELL, A. (1909). *Health Morals and Longevity*. Bristol : John Wright & Sons, Ltd. 229 pp., 22 x 14 cm. Cloth.

A semi-popular work.

LEITH, R. F. C., and FLEMING, R. A. (1908). *Husband's Practice of Medicine* designed for the use of Students and Practitioners. Sixth Edition, re-written and enlarged. Edinburgh : E. & S. Livingstone, 15, Teviot Place. 1141 pp., 19 x 12 cm. Bound cloth.

This well-known students' book has been practically re-written. The bulk of the book is from the pen of Prof. Leith, whilst Dr Fleming is responsible for the parts dealing with diseases of the Nerves and treatment. An excellent index materially enhances the value of the work.

PERYASSÚ, A. G. (1908). *Os Culicídeos do Brazil*. Trabalho do Instituto de Manguiinhos. Rio de Janeiro : Typographia Leuzinger. 407 pp., numerous figures and a map.

The work gives a description of the mosquitoes of Brazil, their structure, biology, and classification. There are many original illustrations.

SIMPSON, W. J. R. (1908). *The Principles of Hygiene as applied to Tropical and Sub-tropical Climates*. London : John Bale, Sons & Danielsson, Ltd., Oxford House, Great Titchfield Street, W. 396 pp., 98 figs., 22 x 15 cm. Price 15/- net. Cloth.

The author, who is Professor of Hygiene at King's College, London, and Lecturer in Tropical Hygiene at the London School of Tropical Medicine, has had a great deal of personal experience, through years of residence in the tropics, of the matter of which he writes. The book will be welcome to students of tropical medicine and hygiene since there has long been a want for a book of this character.

WHITE, F. F. (1908). *Infected Ears* (Intrameatal Treatment). London : Yellon and Mansfield, The Celtic Press, 43, Chancery Lane, W.C. 100 pp., numerous figures, 18 x 13 cm. Price 5/- net. Cloth.

The book contains a record of three years' experience of otectomy and naturally is mainly intended for otologists.

#### BROCHURES.

BURCKHARDT, A. (1908). *Demographie und Epidemiologie der Stadt Basel während der letzten drei Jahrhunderte 1601-1900*. Leipzig : Verlag von Carl Beck. 111 pp., 20 tables, 2 charts. Price 6 Marks.

The author, who is Professor of Hygiene in the University of Basel, presents us with an account of the Demography and Epidemiology of the city of Basel, covering a period of three centuries. There are few cities whose records are available for such a study, consequently this publication is one of exceptional interest. A good bibliography, numerous statistical tables and a chart of births and deaths for the period 1601-1900 conclude the work.

GLÜCK, L. (1908). *Die Lepra tubero-anaesthetica vom klinischen Standpunkte geschildert*. Reprinted from *Lepra. Bibliotheca internationalis*, Vol. VIII., Nos. 1 & 2, 80 pp., 11 figs. Leipzig : J. A. Barth. Price, unbound, 4 Marks.

A clinical study of tubero-anaesthetic leprosy based on the late author's

study of ten cases at Sarajevo. The author was the first to discover leprosy in Bosnia and Herzegovina, but he was not allowed to publish the facts for the reason that the Vienna authorities thought Bosnia, "the land of tourists," would be injured thereby. The author maintains, as against Hansen and others, that tubero-anaesthetic leprosy does exist as a distinct clinical form of the disease.

MACILWAINE, S. W. (1908). *The Future of Medicine*. London: P. S. King & Son, Orchard House, Westminster. 44 pp. Price 1/- net.

This pamphlet, which is excellently written, is intended "to point the way that the medical profession must take in order to realize the promise of the future." It is intended for medical men and intelligent laymen. The subject-matter is grouped under the following headings: The use and misuse of the word "disease"—the diseases of intrinsic origin—pathology and the science of medicine—medical education—the need of organization and method in the study of disease.

## REPORTS.

*Annual Report of the Sanitary Commissioner with the Government of India, for 1907*, with Appendices and returns of sickness and mortality among European troops, native troops and prisoners in India, for the year. 146 pp., 53 tables. Calcutta, 1908: Superintendent of Government Printing, India. Price 4/6. Boards.

ELKINGTON, J. S. C. (1908). *Report on the Work of the Medical Branch Education Department, Tasmania*. Tasmania: John Vail, Government Printer, Hobart. 15 pp.

FOWLER, C. E. P. (1908). *Report on Malaria Investigations in Mauritius from November, 1907, to February, 1908*. London: Messrs Harrison & Sons, St Martin's Lane. 82 pp., 4 maps and many plates.

GOSIO, B. (1906). *Studi sulle bioreazioni dell' arsenico, tellurio e selenio a loro applicazioni pratiche*. Ministero dell' Interno. Direzione Generale della Sanità Pubblica. Roma: Tipografia delle Mantellate. 225 pp., 4 plates.

GOSIO, B., and PALADINO, A. (1907). *Contributo alla diagnosi della Pellagra con particolare riguardo ai suoi stadi iniziali*. Ministero dell' Interno. Direzione Generale della Sanità Pubblica. Roma: Tipografia delle Mantellate. 86 pp.

HILL, E. (1908). *Colony of Natal. Report of the Health Officer for the year ended 31st December, 1907*. Pietermaritzburg: "Times" Printing and Publishing Co., Ltd. 37 pp. Price 2/9.

CALMETTE, A. (1909). *Recherches sur l'épuration biologique et chimique des eaux d'égout effectuées à l'Institut Pasteur de Lille et à la Station Expérimentale de la Madeleine*. Vol. iv., 214 pp., with numerous figures. Paris: Masson & Cie.

*Fourth Annual Report of the Henry Phipps Institute for the Study, Treatment, and Prevention of Tuberculosis*, February 1, 1906, to February 1, 1907. Edited by J. Walsh, A.M., M.D., contains the following papers:—Clinical and Sociological Report of the Year, by L. F. Flick, pp. 5-107.—A Study of the Blood in Pulmonary Tuberculosis, by F. A. Craig, pp. 108-119.—Albuminuria and Casts in Pulmonary Tuberculosis, by C. M. Montgomery, pp. 120-157.—Laryngological Report of the Year, by H. J. Off, pp. 158-162.—The Importance of the Upper Respiratory Tract in the Etiology of Cryptogenetic Infections, especially in Relation to Pleuritis, by G. B. Wood, pp. 163-202.—Tuberculosis of the Bones and Joints, by W. W. Cadbury, pp. 203-223.—Tuberculosis of the Lymphatic Glands

and Skin, by J. D. Blackwood, pp. 224-237.—Neurological Report of the Year, by D. J. McCarthy, pp. 238-264.—The Pleura in Pulmonary Tuberculosis, by W. B. Stanton, pp. 265-289.—Comparison of the Pathological Findings with the Recorded Clinical Signs in Nine Cases of Tuberculosis of the Lungs, by J. Walsh, pp. 290-315.—Pathological Report of the Year, by C. Y. White, pp. 316-377.—Bacteriological Report of the Year, by J. W. Irwin, pp. 378-389.—The Relation of the Pneumococcus to Hemorrhage in Tuberculosis, by J. Walsh, pp. 390-395.—Comparison of Results at White Haven Sanatorium and Phipps Institute, by J. Walsh, pp. 396-402.—Training School for Nurses of the Henry Phipps Institute, by G. B. Wood, pp. 403, 404.

*Report of the Board of Health on Leprosy in New South Wales for the year 1907.*

30 pp. Sydney, N.S. Wales, 1908: William A. Gullick, Government Printer.

*Report of the Board of Health on Plague in New South Wales, 1907.*—1. On a Seventh Outbreak of Plague at Sydney, 1907, by J. A. Thompson, M.D.—2. On an Outbreak of Plague at Kempsey, Macleay River, 1907, by R. J. Millard, M.B. Sydney: William Applegate Gullick, Government Printer. 67 pp. Price 2/6.

ROSS, RONALD (1908). *Report on the prevention of Malaria in Mauritius.* London: Waterlow & Sons, Limited. 202 pp., 25 photographs.

THEILER, A. (1908). *Report of the Government Veterinary Bacteriologist, 1906, 1907.* Pretoria: Printed at the Government Printing and Stationery Office. 264 pp.

*Third Scientific Report on the Investigations of the Imperial Cancer Research Fund*, by Dr E. F. Bashford. London: Printed and published by Taylor & Francis, Red Lion Court, Fleet Street, E.C. 484 pp. Price 15/- Boards.

Contains the following papers:—The Ethnological Distribution of Cancer, by E. F. Bashford, pp. 1-25, 8 figs.—On the Occurrence of New Growths among the Natives of British New Guinea, by C. G. Seligmann, pp. 26-40, 8 figs.—The Zoological Distribution of Cancer, by J. A. Murray, pp. 41-60, 19 figs.—On the Occurrence of Heterotypical Mitoses in Cancer, by E. F. Bashford and J. A. Murray, pp. 61-68, 15 figs.—Spontaneous Cancer in the Mouse; Histology, Metastasis, Transplantability, and the Relations of Malignant New Growths to Spontaneously Affected Animals, by J. A. Murray, pp. 69-114, 43 figs.—The Haemorrhagic Mammary Tumours of Mice, with Results of Research into Susceptibility and Resistance to Inoculation, by E. Gierke, pp. 115-145, 20 figs.—The Effects of Surgical Interference with the Blood Supply on the Growth of Transplanted Carcinoma and Sarcoma, by W. H. Bowen, pp. 146-158, 6 figs.—A Transplantable Squamous-Celled Carcinoma of the Mouse, by J. A. Murray, pp. 159-174, 13 figs.—Contributions to the Study of the Development of Sarcoma under Experimental Conditions, by M. Haaland, pp. 175-261, 100 figs.—General Results of Propagation of Malignant New Growths, by E. H. Bashford, J. A. Murray, M. Haaland, and W. H. Bowen, pp. 262-283, 24 figs.—The Experimental Analysis of the Growth of Cancer, by E. F. Bashford, J. A. Murray, and W. H. Bowen, pp. 284-314, 12 figs.—The Natural and Induced Resistance of Mice to the Growth of Cancer, by E. F. Bashford, J. A. Murray, and W. Cramer, pp. 315-340, 1 fig.—The Nature of Resistance to the Inoculation of Cancer, by B. R. G. Russell, pp. 341-358, 10 figs.—Resistance and Susceptibility to Inoculated Cancer, by E. F. Bashford, J. A. Murray, and M. Haaland, pp. 359-397, 20 figs.—Report on a Study of the Variations in the Secretions of Hydrochloric Acid in the Gastric Contents of Mice and Rats, as compared with the

Human Subject, in Cancer, by S. M. Copeman and H. W. Hake, pp. 398-419, 14 tables.—Glycogen and Fat in Malignant New Growths of the Mouse, by M. Haaland, pp. 420-426, 2 figs.—The Gaseous Metabolism in Rats inoculated with Malignant New Growths, by W. Cramer, pp. 427-434, 2 tables.

### NEW JOURNALS.

*Malaria. International Archives.* Edited by Dr C. Mense (Cassel). Vol. I., No. 1, Oct. 1908, 88 pp., 2 pl. and 2 charts. Leipzig: Johann Ambrosius Barth, Dörrienstr. 16; London: Williams & Norgate; New York: G. S. Stechert & Co.; Paris: Librairie Charles Delagrave; Rome: Loescher & Co. Annual subscription, £1.

This new quarterly Journal will publish papers in English, German, French and Italian, and will contain reviews of current literature and a bibliography which will be made as complete as possible. The opening number contains original papers by Celli, Lemaire and Dumolard, Nocht, Gonter and v. Berenberg-Gossler, and Mollow. Two excellent coloured plates by Gonter and v. Berenberg-Gossler illustrate Malaria parasites of monkeys.

*Pathologica. Rivista Quindicinale.* Vol. I., No. 1, 24 pp. (15. xi. 1908). Published by P. Foà, G. Galeotti and L. Griffini, the chief Editor being Mario Segale. Geneva: The Editors, Instituto di Patologia Generale; the Publishers, Via Agostino Bertani, 5. Annual subscription in Italy 15 Lire, abroad, 20 Lire.

A bi-monthly periodical, on whose title-page appear the names of the leading pathologists of Italy. The first number contains short papers by Foà, Barbacci, Donati and Satta, Parodi, Centanni, and Mya, the concluding pages containing reviews of pathological papers.

*Sleeping Sickness Bureau. Bulletin.* Issued under the direction of the Honorary Managing Committee. Editor: The Director of the Bureau. No. 1. 51 pp. London: Sleeping Sickness Bureau, Royal Society, Burlington House, W.

The first number of the Bulletin appeared in Oct., 1908, since which date there have appeared Nos. 2-5 (April, 1909). The Bulletin contains abstracts of papers dealing with sleeping sickness as well as bibliographies, etc. The publication promises to be most useful.

*Wasser und Abwasser. Zentralbl. f. Wasserversorgung u. Beseitigung flüssiger u. fester Abfallstoffe, Bd. 1, No. 1 (15 Jan., 1909), 40 pp.* Edited by Schiele, A., and Weldert, R. Annual subscription 30 M. Sample copies sent on application to the Publishers: Gebrüder Borntraeger, Crossbeerenstr. 9, Berlin, S.W. 11.

This new monthly periodical deals entirely with matters relating to water supply and sewage disposal. The opening number contains an original article by Dr A. Schiele, the chief editor, who is an Engineer, and numerous reviews of recent American, British, French and German literature follow.

*Zeitschrift für biologische Technik und Methodik.* Edited by Dr M. Gildemeister (Privatdozent des Physiologen in Strassburg i. E.). Vol. I., No. 1, May, 1908. Strassburg: K. J. Trübner. Published at irregular intervals in accordance with the material which is available. Subscription, 15 Marks per volume of about 480 pp.

This new Journal will contain short original papers (in German, if necessary they will be translated into German), notes and reviews. The subjects treated will relate chiefly to the physiology of animals and plants; physiological chemistry,

bacteriology, chemistry of fermentation, pharmacology, experimental pathology and psychology, experimental morphology, embryology and heredity. The opening number contains papers by J. R. Ewald, W. Berndt, T. Thunberg, W. Roux, H. Zwaardemaker, O. Langendorff, J. K. R. W. Salomonson, F. Mandel, M. Gildemeister, O. Weiss, G. Joachim and E. Herrmann, besides numerous reviews, etc.

*Zeitschrift für Immunitätsforschung und experimentelle Therapie.* I Teil : *Originale.* Edited by E. Friedberger (Berlin), R. Kraus (Wien), H. Sachs (Frankfurt a/M), P. Uhlenhuth (Gr.-Lichterfelde-Berlin). Vol 1., Nos. 1-6=800 pp. Price, per volume, 18 Marks. Jena: Gustav Fischer. Editorial communications to be sent to Prof. Friedberger, Dorotheenstr. 34a, Berlin N.W. 7.

The first part of this Journal contains only original papers and the numbers will be issued as rapidly as sufficient material has been received to fill a number. Authors will receive 50 reprints of their papers free, besides an honorarium of 40 Marks per sheet. The following list of papers which have appeared in the opening numbers sufficiently indicate the very valuable contents of this new and very important Journal:

*Heft 1 (21 Dec., 1908).*

EHRlich, P., Zur Einführung, p. 1.—LEVIN, E. L., Ueber passive Immunität (Mit 22 Kurven in Text), p. 3.—STRENG, O., Existieren echte Antialexine (Antikomplemente)? p. 28.—PETTERSSON, A., Ueber hitzebeständige, alkohollösliche, bakterizide Substanzen der Leukocyten, p. 52.—MÜLLER, P. T., Einige Versuche über die Rolle der Bakterienlipoiden bei der Phagocytose, p. 61.—ROEHL, W., Ueber Trypanosan, p. 70.—SCHWARZ, O., Ueber den Einfluss künstlicher Aenderungen im Bakterienprotoplasma auf dessen agglutinogene Fähigkeiten, p. 77.—HAENDEL and SCHULTZ, W., Beitrag zur Frage der komplement-ablenkenden Wirkung der Sera von Scharlachkranken, p. 91.—KRAUS, R., and SCHWONER, J., Ueber Beziehungen der Toxolabilität und Toxostabilität der Antitoxine zu deren Heilwerte, p. 103.—UHLENHUTH and MANTEUFEL, Chemothapeutische Versuche mit einigen neueren Atoxylpräparaten bei experimentellen Spirochätenkrankheiten mit besonderer Berücksichtigung der Syphilis, p. 108.—SACHS, H., and RONDONI, P., Beiträge zur Theorie und Praxis der Wassermannschen Syphilisreaktion, p. 132.—v. EISLER, M., and v. PORTHEIM, M., Ueber ein Hämagglutinin im Samen von Datura, p. 151.—FRIEDBERGER, E., and SACHS, F., Ueber die Einwirkung von Arsenpräparaten auf den Verlauf der Lyssainfektion (Virus fixe) beim Kaninchen, p. 161.

*Heft 2 (27 Jan., 1909).*

RÖMER, P. H., Ueber die intestinale Resorption von Serumantitoxin und Milchantitoxin, p. 171.—ZANGGER, H., Die Immunitätsreaktionen als physikalische, speziell als Kolloid-Phänomene, p. 193.—WILKENO, M., Ueber Immunisierung mit Kot und über das Verhalten des Inhaltes verschiedener Darmpartieen gegen Kotpräzipitin und Serumpräzipitin. Erste Mitteilung, p. 218.—FUKUHARA, Ueber den Zusammenhang alkoholischer (hämolytisch, bakterizid, wirkender) Substanzen der Organe mit den normalen und immunisatorisch erzeugten Antikörpern, p. 224.—VAUGHAN, V. C., Protein Sensitization and its Relation to some of the infectious Diseases. With 5 Charts, p. 251.—CALMETTE, A., Die Tuberkuloseinfektion und die Immunisierung gegen die Tuberkulose durch die

Verdauungswege, p. 283.—VON EISLER, M., Ueber den Zusammenhang der Wertigkeit und Avidität bei Bakterienagglutininen, p. 297.—KRAUS, R., v. EISLER, and FUKUHARA, Ueber Adsorption des filtrierbaren Virus, p. 307.—SCHATLOFF, P., and ISABOLINSKY, M., Untersuchungen über die Wassermann-Neisser-Brucksche Reaktion bei Syphilis, p. 316.—SELIGMANN, F., Zur Kenntnis der Wassermannschen Reaktion, p. 340.—TERRUCHI, Y., Vergleich der Hämolyse durch Natronlauge und Vibriolysin in verschiedenen isotonischen Medien, p. 351.

*Heft 3* (10 Feb., 1909).

RÖMER, P. H., Ueber das Vorkommen von Tetanusantitoxin in Blute normaler Rinder, p. 363.—ATKIN, E. E., Spontaneous Agglutination of Horse Erythrocytes suspended in Sodium Chloride Solution. A Contribution to the Haemolytic Technique, p. 387.—RAUBITSCHEK, H., and RUSS, V. K., Ueber entgiftende Eigenschaften der Seife, p. 395.—KRUSCHILIN, A. W., Ueber die Wirkung des Alkohols auf die Tätigkeit der Phagocyten, p. 407.—STERN, M., Eine Vereinfachung und Verfeinerung der serodiagnostischen Syphilisreaktion, p. 422.—LANDSTEINER, K., and v. RAUCHENBICHLER, R., Ueber das Verhalten des Staphylolysin beim Erwärmen, p. 439.

*Heft 4* (20 Feb., 1909).

BASHFORD, E. F., MURRAY, J. A., and HAALAND, M., Ergebnisse der experimentellen Krebsforschung, Mit 41 Figuren in Text, p. 449.—BAIL, O., and TSUDA, K., Versuche über bakteriolytische Immunkörper mit besonderer Berücksichtigung des normalen Rinderserums, p. 546.

*Heft 5* (11 March, 1909).

GEWIN, J., Zur Frage des Ambozeptor gehaltes des Säuglingsblutes, p. 613.—BREINI, A., and NIERENSTEIN, M., Zum Mechanismus der Atoxylwirkung, p. 620.—ROEHL, W., Heilversuche mit Arsenophenylglycin bei Trypanosomiasis, p. 633.—EISNER, G., Untersuchungen über die antifermentative, besonders die antityryptische Wirkung des Blutserums, p. 650.—PICK, E. P., and YAMANOUCHI, T., Chemische und experimentelle Beiträge zum Studien der Anaphraxie, p. 676.—KRAUS R., and VOLK, R., Zur Frage der Serumanaphraxie, p. 731.—AGAZZI, B., Ueber den Einfluss einiger Arsenpräparate auf die Intensität der Bildung von bakteriellen Antikörpern (Agglutininen) beim Kaninchen, p. 736.

*Heft 6* (25 March, 1909).

THOMSEN, O., Ueber die Spezifität der Serumanaphraxie und die Möglichkeit ihrer Anwendung in der medikoforensischen Praxis zur Differenzierung von Menschen- und Tierblut (in Blutflecken, etc.), p. 741.—UHLENHUTH, P., Bemerkung zu vorstehender Arbeit von O. Thomsen, p. 770.—BAIL, O., and TSUDA, K., Beobachtungen über die Bindung bakteriolytischer Immunkörper an Vibrionen, p. 772.

*Zeitschrift für Immunitätsforschung und experimentelle Therapie.* II. Teil: *Referate* (Centralblatt für die gesamte Immunitätsforschung und experimentelle Therapie). Vol. I., No. 1, 104 pp. Price, per volume, 22 M.

The second Part of the Journal contains numerous reviews. Parts will be issued at irregular intervals. Authors of reviews will receive 75 M. honorarium per sheet; fifty sheets will form the volume. Editors and Publishers the same as for Part I.

## HOUSE FLIES AS CARRIERS OF DISEASE.

By J. T. C. NASH, M.D., D.P.H.,

*County Medical Officer of Health for Norfolk.*

### TABLE OF CONTENTS.

	PAGE
(I) What is the "House-fly" . . . . .	141
(II) The biology of <i>Musca domestica</i> as studied by the Author and others. Breeding and Dietary; Range of Flight; How to rear house-flies in captivity.	142
(III) The relation of <i>Musca domestica</i> to Disease. Theoretical and Experimental considerations. Cholera, Typhoid, Epidemic diarrhoea, &c. . . . .	148
(IV) Epidemiological relation between flies and epidemic diarrhoea . . . . .	151
(V) References to literature . . . . .	160
(VI) Summary and methods of Prevention . . . . .	162

### (I) What is the House-fly?

ALL observers agree that *Musca domestica* comprises over 90 per cent. of the flies found in houses; my own observations show 97 per cent. *M. domestica* is most easily distinguished by the venation of its wings, the fourth vein being bent up at an angle, and by the four longitudinal black stripes on the thorax. It is thus easily distinguished from *Homolomyia canicularis*, another somewhat smaller fly which frequents houses and forms the bulk of the remaining 3—10 per cent.

A biting fly, *Stomoxys calcitrans*, is occasionally found in houses, but rarely. In the course of a six weeks daily investigation in my own kitchen I have come across only one specimen, which was readily recognised by its proboscis. During the past summer I have been at least twice bitten by this species in the country out of doors. Mr Hewitt says that in the winter it is often seen in stables and cow-sheds and many can confirm his statement.

*Stomoxys* has a pointed proboscis; it is very like the domestic house-fly in general appearance, but close inspection reveals the needle-like proboscis and a spotted abdomen.

It may be thought that the anatomy and life-history of the house-fly had never been investigated until recently. This however is not the case. I am indebted to Mr E. E. Austen for having drawn my attention to a copy of a rare work by Gleichen and Keller (1790) which came into the possession of the British Museum recently. It comprises several beautiful plates illustrating the anatomy and metamorphic life-history of the house-fly. It is however so rare, that it is doubtful where another copy could be consulted. Apparently until quite recently no work on the subject of house-flies had been published in England, but in America Packard (1874) and Howard (1902) published their respective researches.

(II) **The biology of the House-fly as studied by the Author and others.**

(1) *Breeding Places and Dietary.*

In 1902 and later years I was so certain from my own observations that flies bred in deposits of house refuse and manure and other organic fermenting refuse, that I actually mentioned such in reports as breeding places, thinking it a well-known fact; but apparently it was not until Newstead (1907) of Liverpool, and Gordon Hewitt (1908) of Manchester studied the habits of *Musca domestica* that this became an established fact in modern scientific circles in England. I look upon refuse heaps of recent origin, not only as fly-breeding deposits, but also as incubators for infective and putrefactive germs, including diarrhoea-causing organisms. Inasmuch as pernicious germs—and insects adapted for carrying them—are bred in these refuse heaps, such heaps must be reckoned among some of the important and principal sources whence dangerous germs are carried to food during summer and autumn. Newstead agrees that outside a town the enormous collections of house refuse must be reckoned a principal breeding place of flies. Both Hamer (1908) and Newstead incriminate accumulations of manure at wharfs, and the former says that in London, horse manure pre-eminently stands out as the chosen breeding ground for house-flies.

Newstead says that ash pits emptied at intervals of about 14 days may be considered as temporary breeding places, the period between the removals being as a rule too short for the fly to complete its life cycle. But ash pits in some parts of the country are made of such a size that they are not emptied for months. The vegetable matter fermenting in them may then be classed, in my opinion, as a fairly permanent breeding

place. Moreover bricked ash-pits are liable not to be thoroughly cleaned out when emptied even once a fortnight or oftener, and fly larvae may be left behind by the scavenger and so be able to complete their life cycle. In any case the larvae are not destroyed by the scavenger but carried away to be dumped on a refuse heap, there possibly to complete the cycle and give rise to many more generations of flies and larvae in countless numbers.

I may here refer to another experience of my own. In 1904, I gave evidence in favour of an Urban District Council which wished to convert some bin privies into water closets. I had found the spaces around the moveable excreta boxes in these privies simply swarming with fly larvae, and on pointing out to the court the danger of disease being carried from the privies by flies, the case was speedily decided in favour of the Urban Council.

Newstead has shewn that the dietary of the larvae of *Musca domestica* is almost exclusively that of moist decaying vegetable matter.

He found that *horse manure* and *spent hops* are the most favoured, but the larvae revel also in rotten flock-beds, straw-mattresses, old cotton garments, rotten socks, and waste paper, and are especially partial to the dirty beddings from rabbits and guinea-pigs. They feed also on bread, decayed fruits, and vegetables, and on the excreta of all animals into whose dietary vegetable food enters.

Newstead's investigations not only determined the chief breeding places but brought to light many new and interesting facts relating to the food of the larval stages. "Stable middens broadly speaking are the chief breeding places, the larvae in countless thousands revelling in the heat produced by fermentation."

I recognised this fact years ago and instructed the Sanitary Inspectors under me to pay the utmost attention to mews, and to compel the frequent removal of manure.

Mr E. E. Austen kindly allowed me to take a note of an unusual breeding place which came under his notice in 1908. Messrs Siemen Bros. and Co. communicated with Mr Austen concerning what they called "worms" found in some *rubber* which had been washed on July 24th and passed through washing rollers under great pressure with streams of hot water.

The rubber so treated was then suspended in a drying room at a temperature of 100° F. On July 27th this rubber was swarming with "maggots" which Mr Austen found to be larvae of the house-fly apparently of full size. The time for the development of these full sized larvae from the egg stage could not have exceeded *three days*, which

is the shortest time on record and indicates that the temperature of 100° F. with plenty of moisture was very favourable.

I have seen pupae by the thousand in stored house refuse. Mr Newstead has seen as many as 78 females laying their eggs in one small area. Each fly lays over 100 eggs, and the favoured spots are amongst fermenting vegetable matter or in refuse that is likely to ferment. In warm weather with abundance of fermentable substances to feed on, the larvae rapidly mature.

A single fly at the commencement of summer may quite possibly give rise to millions of descendants before the first frosts of autumn cut short their multiplying career. Packard estimates the probable number of descendants of one fly at 125,000,000.

Frost is the greatest enemy to flies, and fortunately for Great Britain cold speedily reduces the enormous battalions of flies which come into existence during the warm weather and autumn.

#### (2) *The Periods of Metamorphic Change.*

All observers agree that under exceptionally favourable circumstances the whole life-history of the fly, from egg to perfect insect, may take only eight days, about five for the larval stage, and about three for the pupal stage. Under less favourable conditions the changes are slower, and for average conditions from ten to fourteen days elapse.

In November and December, in a kitchen, I found that the larval stage occupied 3 weeks. After the pupal stage had lasted 7 days I placed a pupa in a match box on the kitchen mantel-shelf and it hatched out in 40 hours at the increased temperature of this position in the room. Prior to this the pupae were on a shelf about 8 ft. from the fireplace.

Newstead and Hewitt say that the liberation of the fly from the pupa is accomplished by the fly within breaking away the anterior end of the pupa case by means of an inflated frontal sac situated in the anterior part of the head between the eyes. Packard says this sac is distended with blood and forms a bladder-like expansion, trapezoidal in outline, equal in bulk to the rest of the head.

Griffith (1908), says "it is a curious sight to watch the efforts of these flies to get out of their shells; the head is distended until it looks like bursting and the fly makes most frightful grimaces."

The difference between male and female fly is easily observed by noting the space between the large compound eyes. In the male the head appears to be almost all eyes. In the female there is a space

between the eyes equal to one third of the diameter of the head. In this space are three simple eyes or ocelli arranged in the form of a triangle between the compound eyes.

The larval stage corresponds to the period of growth; no insects grow after they have attained the fly stage. There may be small flies and larger flies of the species—these variations in size depend on the conditions which affected the larval growth. Sometimes extraordinarily diminutive specimens of flies emerge when breeding takes place under artificial conditions, more especially if the temperature fluctuates and averages under 60° F. Under natural conditions the length of an ordinary house-fly (*M. domestica*) is about a quarter of an inch.

The larvae of *M. domestica* can be easily distinguished by means of the posterior stigmatic plates which are situated on the square terminal segment. The openings in each posterior stigmatic plate are in the form of two very sinuous fissures which are characteristic.

The larvae of *Stomoxys calcitrans* are similar in general appearance to the larvae of *M. domestica*; they also breed in excremental matters and decaying vegetable matter in a fermenting condition. Mr Gordon Hewitt states that *H. canicularis* prefers the old style midden to all other breeding places. Its larvae are easily distinguished from those of *M. domestica*, the segments of the body bearing spine-like processes.

### (3) *The Range of Flight of Flies.*

A very important point is, how far can flies travel?

From my own observations, I find that from a large breeding place such as tons of house refuse on a brickfield they will travel in countless battalions to the nearest houses which may be two hundred or three hundred yards distant. In warm weather they travel further, and they will be found in considerable but decreasing numbers in houses within one third to half a mile distant. *Each street and terrace of houses forms a place of arrestment*, provided there is abundance of pabulum whether in the nature of filth or ordinary articles of human diet. Where few or no houses intervene, flies in large numbers will travel considerable distances—even over half a mile.

The worst affected house I have seen was a good middle class house standing alone in its own grounds in an open district. In this house the ceilings, the walls, the tables, the beds, were literally black with these pestiferous insects. Two young children under three years of age had suffered from severe diarrhoea, to which the younger, an infant,

of only six months, had already succumbed ; the older child's life was saved only by immediately sending it away. Casting about for an explanation of the extraordinary plague of flies, I found that this house was situated between four farms—each within a quarter to half a mile of the house. At most farms large quantities of manure and other organic decaying matter are generally to be found, but I think the principal source of the mischief in this particular instance was that at one of these farms, only a quarter of a mile distant, a large heap of recent house refuse had been accumulating by daily additions of several cartloads.

A further personal experience as to the distance flies may travel is quite recent. At the beginning of October 1908 I went into a new house. I was horrified to find that the kitchen was daily invaded by hordes of flies. I knew that so many flies meant objectionable deposits somewhere near. On investigating the neighbourhood I saw a large heap of manure in the centre of a large vegetable field about three hundred yards away from my house, and two or three other such deposits in adjacent fields. Further on I found a piggery, but perhaps most important of all, I think, I found several large collections amounting in all to hundreds of tons of house refuse within a radius of a quarter to half a mile. My neighbours were troubled not only with flies in their kitchens but those nearer the deposits of filth found the flies a nuisance even in their bedrooms.

#### (4) *How to rear House-Flies in captivity.*

I have reared the larvae of *M. domestica* under an ordinary glass on bread, on pear, potato, and banana peelings, and old paper. Moist bread mash or boiled rice is sufficient.

The following is a simple and fairly satisfactory method of breeding the larvae in a room warmed by a fire, such as a kitchen, or in a laboratory which is maintained at a temperature of about 60° F.

Place on a clean glass slab or plate several pieces of stale moistened bread, and one or two shreds of old newspaper all heaped up for two or three inches, and cover over with a long pint tumbler.

Catch in the palm of the hand, by a swift sweep of the hand, some flies in a warm room, and for convenience of transferring to under the glass, first of all throw them into a flat dish of water. This partly stuns them and wets their wings, at any rate enough to enable them to be taken up one by one. Each individual fly can then be examined to

determine the sex and species. In this way specimens of *M. domestica*, male and female, can be transferred to under the glass. From day to day add a small piece of moistened stale bread to the collection. The flies themselves by their presence aid in the fermentation of the bread mash, chiefly no doubt by means of their bacteria-laden legs.

Should any piece of bread show signs of a mould or Sarcina growth at once remove the piece, as these forms of growth appear to be hurtful to the larvae. In the course of a few days some flies' eggs will be observed if carefully looked for. They are about 0·5 millimetre in length and about 0·15 millimetre in breadth.

The eggs are laid in heaps, sometimes end to end. In a few hours the eggs become minute larvae which rapidly grow in size and they can often be seen wandering up and down the sides of the glass. The larvae leave behind them a slimy trail, particularly when they have grown larger and fatter. It is therefore advisable to occasionally remove the glass, and after carefully replacing the larvae on the decaying bread heap, to thoroughly wash the glass before replacing it. The larval stage of existence varies in length according to the temperature.

A most lively incident unfortunately deprived me of some of my best larvae, and seems to suggest that purely animal fat is obnoxious to the larvae of the ordinary house-fly, which thus differs *in toto* from the larvae of *Calliphora erythrocephala*, the blue bottle fly.

One evening I placed under two glasses which already contained some vegetable decaying matter and a goodly brood of fly larvae, some pieces of a cream cheese which was beginning to turn rancid.

The effect was most extraordinary. In a few minutes I observed that the larvae which had found the conditions hitherto obtaining entirely to their liking, were bent now on making their escape with all possible speed.

They evidently could not abide the presence of the rancid cheese. Not wishing to lose my larvae and being also unwilling to cut short what promised to be an interesting experiment, I surrounded the glasses about one inch from the margin with a mixture of mustard and salt, but the larvae preferred to brave even these dangers and discomforts and those of the cold night air, to remaining within touch or smell of the cream cheese. It was an extraordinary exodus, like an army in full flight.

What was the explanation of this curious phenomenon? Either some acrid emanation of a toxic nature from the cheese, or a very acute and particular sense of smell on the part of the larvae, or an instinct

that there was near them a fatty substance which might obstruct their breathing tubes; at any rate something urged these larvae to leave their warm fermenting natural abode with its abundance of food hitherto to their liking, and brave the perils of the unknown cold outside world. I had previously observed that a fly would expire almost at once if thrown into warm grease-containing water.

### (III) The Fly in relation to Disease.

Looking at a fly under a low magnifying power it is possible for a bacteriologist to at once appreciate how dangerous a carrier of bacteria a single fly can be. Its body is covered with bristles but more particularly its legs, which are composed of five segments, the last having no fewer than five joints.

Each one of the numerous bristles on the fly's head, body and legs, is capable of carrying hundreds of germs. When we examine the wings of a fly under a somewhat higher power, magnifying say 50 diameters, we find that even the margins of the wings are beset with pointed scales, each capable of bearing scores of bacteria and particles of dust.

I was surprised to see a statement by one writer<sup>1</sup> that he thought infection could not cling to a fly for any length of time because it was always cleaning itself. Those among practitioners who practice surgery know how difficult it is, in fact impossible,—after even the most vigorous washing and scrubbing of the hands with the aid of a brush, and anti-septic solutions, applied even for a considerable time,—to rid the human hand of all germs; and consequently in any delicate operation the careful surgeon puts on sterilised rubber gloves over his hands even after he has washed and scrubbed them. It is simply impossible for a fly to clear itself quickly of all objectionable germs as the experiments I shall quote will show.

Further evidence of the fly's inability to rid itself of germs is the fact that large numbers of flies die in the autumn of a disease caused by a fungus known as the *Empusa muscae*—the mycelial threads of which penetrate and destroy the internal organs of the fly, and then shoot out numerous spore-bearing filaments from the surface of its body. In the autumn it is quite common to notice a fly on the wall in apparently a natural position; on closer inspection you find it is dead, and looking more closely you notice that its abdomen is distended and

<sup>1</sup> *Public Health*, May 1908.

the segments obtrusively defined by white rings of mycelia. Often the whole fly is surrounded by a white cloud of spores.

Numerous experiments have been made by various observers to prove that the fly is a carrier of bacteria to human food. I have experimented myself in this direction and it may therefore not be out of place to here record one or two of my own experiments which I endeavoured to carry out on ordinary every-day lines so as to be able to judge of natural conditions, and allow for symbiosis, antagonism of bacteria, &c., such as occur in nature.

In July 1905 I isolated typical excretal *Bacillus coli* from a fly caught in a ward in which were a few typhoid patients.

I have recorded elsewhere (1906) how I caught a single fly in a hospital ward and put it into a tube of fresh sterile broth—then kept the broth at a blood heat, secure from any other contamination for 15 hours. At the end of this time the broth was converted into a stinking fluid teeming with myriads of germs including large numbers of intestinal bacteria. If a single fly at a warm temperature can change a good wholesome broth into a stinking putrid liquid it is easy to understand how even one or two flies immersed in a jug of milk in a warm room will render that article of food a most dangerous poison in a few hours. According to the numbers of polluting flies so will the relative pollution of such milk vary. Hence, when flies are very abundant and are allowed free access to milk (whether cow's or condensed) such milk becomes very rapidly polluted. Although there is much truth in the fact that milk under ordinary conditions contains thousands of germs per cubic centimetre, particularly in the summer weather, yet I am of opinion that, apart from dirty conditions of milking and storage, the majority of such germs are harmless, and will cause souring of the milk prior to developing dangerous qualities as a human food.

If however such milk is polluted with intestinal bacteria, either by dirty methods of milking or by the agency of flies, or otherwise, it may become highly poisonous while yet apparently sound. Flies are in my opinion among the principal contaminating agents of milk by depositing in it diarrhoea-producing organisms. In one epidemic, one of a variety of species of bacteria may be the principal pathogenic agent; and in another, another; or more than one variety of disease germ may exist in combination; but they can be, and, without doubt, are often carried by flies. Without the house the principal breeding places of flies are collections of manure and of house refuse. Other sources of flies are piggeries, stables, slaughterhouses, offensive trade premises,

midden privies, &c. A spot map I prepared in 1904 shewed that such collections or conditions form a sort of nucleus, round which are grouped fatal cases of diarrhoea. Within the house or in a neighbouring dwelling place the excreta from a patient suffering from an intestinal disease forms a potent source of danger through the agency of flies.

#### *Personal Experimental Observations.*

In 1907 I carried out two simple experiments to shew to what extent milk was polluted by flies.

Exp. (1). On a warm day in August 1907, two ordinarily clean saucers were three parts filled from the ordinary morning supply. One saucer was covered over with a clean plate, the other left uncovered. Both were placed on a table in the kitchen where there were some flies; so that both were under identical conditions as to temperature, &c.

After five hours two flies were noticed in the uncovered milk. Bacteriological examinations on exactly similar lines were then instituted as to the bacterial conditions of the covered and uncovered milks.

The comparative results may be put into the form of a diagram. Briefly the experiment shewed that there were more than twice as many bacteria in the milk which had been polluted by the flies as in the other which had been kept covered and protected from flies. On estimating the number of bacteria numerically, this meant that in every c.c. of the fly-polluted milk there were nearly five millions extra bacteria as compared with the protected milk.

The two milks were kept for a further three days, both now kept covered over, to prevent any further contamination.

After a farther twenty-four hours at the temperature of the room the milk which had been previously exposed for 5 hours to the fly pollution had a faint, rotten or putrefactive odour which two days later was very offensive.

The other milk which had all along been protected from flies was clotted, and had the ordinary, not unpleasant, smell of sour milk; but there was no offensive odour whatever.

Exp. (2). On Sept. 12th, 1907, the room temperature being 72° F., two portions of a sample of milk were placed in the same room in a workman's cottage in which flies were observed to be present; one portion was kept covered over, the other left exposed for sixteen hours. At the end of this time the exposed milk had two flies floating in it and eight hours later a slight putrefactive odour was already noticeable,

while the same milk, which had been protected, looked and smelt quite fresh.

Bacteriological examination of these samples shewed that the uncovered milk which had been exposed to flies contained three times as many bacteria capable of growing at room temperature as the milk which had been protected, while at 37° C. although far fewer varieties of organisms were able to develop at this higher temperature, yet there were ten times as many bacteria capable of growing at the body temperature in the fly-polluted milk as in the milk which had been protected from flies. [To state the number of bacteria per c.c. it was found that there were about 500,000,000 more bacteria per c.c. capable of growing in gelatine, at room temperature, and about 618,000 more bacteria capable of growing at blood-heat in the same quantity of exposed or fly-polluted milk as compared with the milk which had been *covered over* during the sixteen hours the other sample had been exposed.]

Other minute portions of the same samples of milk examined for spores under anaerobic conditions revealed gas-forming bacteria in abundance in a very small drop of the fly-polluted milk, while none were present in the same quantity of the covered-over milk.

The importance of covering over milk is amply demonstrated by the experiments I have here detailed. Every fly which settles on the margin of, or falls into, milk adds its evil contribution in the shape of bacteria. It can therefore be readily understood that the greater the number of flies about, the greater the risk of serious pollution. Although many varieties of these bacteria might be swallowed by the *thousand* with impunity, it does not follow that the same impunity results if they are swallowed by the *million*. The experiments are chiefly indication experiments pointing out the gross effects of even only two or three flies, drowned in milk.

It is not difficult to understand from these experiments how in a fly-ridden district diarrhoeal diseases may be in excess unless care is taken to see that food supplies (especially milk) are protected from the attention of flies.

#### (IV) Observations by the Author as to the Seasonal Relation between Flies and English Summer Diarrhoea.

In 1902, I first was struck with the remarkable coincidence between absence of flies and the complete absence of fatal infantile diarrhoea

during the month of August in that year; and again with the almost coincidental advent of flies and of fatal infantile diarrhoea in the following month of September.

A conference of Essex Medical Officers of Health was convened by Dr Thresh on Nov. 12th, 1902, at Leytonstone, to discuss infantile mortality in Essex. I then maintained, that, as had been previously held, it was clear that season and insanitary conditions were important factors in the aetiology of infantile summer diarrhoea; but that, in my opinion, these two factors were merely *indirect*: the surrounding insanitary conditions being in existence all the year round, while seasonal influence obviously played a very important part. But I expressed the opinion that both acted only in "conjunction" and only *indirectly*—by way of the hot weather (that is the factor of *temperature*) being the favourable season for the development and multiplication of *flies* which bred in insanitary refuse deposit under favourable meteorological conditions. Proceeding direct from fermenting and putrefying organic collections, flies deposit in milk fermentative and putrefactive bacteria which multiply very rapidly during the warmth of summer, converting the hand-fed infants' milk into a dangerous chemical poison capable of giving rise to intestinal irritation. The more numerous the flies become, the more numerous are the cases of diarrhoea which occur. Flies being omnivorous and coprophilous in their habits, each fresh case of diarrhoea becomes a possible source of accentuated-in-virulence, diarrhoea-causing, organisms and thus a vicious circle is set going.

These views were again brought forward by me in January 1903 in a contribution to the discussion on Professor Delépine's paper on "The Bearing of outbreaks of Food Poisoning upon the Etiology of Epidemic Diarrhoea" which was read before the Epidemiological Society of London in December 1902. In this later contribution I specifically joined issue with the late Dr Ballard, as regards there being only one specific micro-organism for epidemic diarrhoea whose vital manifestations were dependent upon conditions of season, &c., and suggested that were the simpler and more comprehensive word "organism" substituted for Dr Ballard's "Micro-organism," such "organism" would fit in very well with the life-history of the common house-fly.

The point I even at that time wished accepted was that "flies" were among the chief agents concerned in carrying faecal pollution to milk during the summer months.

I, even at that time, stated my belief that refuse and midden collections in the proximity of *cowsheds* were a source of great danger, to a

lesser degree through dust in dry windy weather and to a very much greater degree through the risk of flies carrying contamination direct from such collections to the milk or even to the udders of the cow or the hands of the milker.

Subsequent to such contamination, time and temperature (as has been emphasised by Professor Delépine) play an important part in the increase of dangerous bacteria—not to speak of fresh contamination by flies and dust in the dairy shops, and in the homes of consumers.

I strongly advocate now, as then, an addition to the important preventive measures formulated in Professor Delépine's paper in order to insure:—"the covering over of all standing milk, so as to absolutely prevent the access of flies."

While I have shewn how milk may be partially contaminated by flies even in the cowshed or dairy, I find myself completely in agreement with Dr Newsholme after several years' close inquiry into infantile deaths from diarrhoea, that *domestic* infection of milk is the most common source of diarrhoea. I go further, and say that it is probably only in houses in which flies are noticeable and where milk (whether fresh or condensed) is left exposed that gross diarrhoeal pollution of milk occurs.

I have given my reasons for this hypothesis in the various reports and papers I have referred to. In 1905 (p. 495) I put them compendiously in a paper on "The Waste of Infant Life." In April 1903 I had laid stress on my strong opinion as to the chief part played by the house-fly in the epidemiology of summer diarrhoea, while it also played in my opinion a part, though a much more subordinate one, in the autumnal rise in the incidence of typhoid fever. In reply to the discussion on this paper I called attention to the fact that I had roughly constructed a curve shewing the average annual London diarrhoea mortality which would be found to correspond with the average relative numerical prevalence of flies.

In my annual report on the health of Southend-on-Sea for the year 1903 I mentioned three results of the cool and wet summer which seemed to be largely responsible for the happy reduction in the loss of infant life in that year, not only in the town dealt with in the report, but throughout the kingdom generally. They were,

- (1) the laying of dust,
- (2) the destruction or inhibition of fly life,
- (3) the inhibition of rapid bacterial growth in milk.

When we consider how largely faecal matter such as horse dung enters into the composition of the dust of our streets we can readily

understand that the laying of dust and the scouring of our streets by heavy rain is a blessing not to be lightly estimated.

The same consideration indicates how much more important than the mere laying of mineral dust is the *efficient* watering and scavenging of streets.

During the year 1903 much useful knowledge was gained as to the agency of flies in the propagation and natural history of certain tropical diseases; while in our own country, I still felt almost like one crying in the wilderness as to the part played by flies in accentuating the high mortality among infants which existed in some districts. [How different now when I feel almost lost in the crowd which proclaims it far and wide.]

The summers of 1902 and 1903 were wet and inimical to fly life. In both years the infant mortality figure was far below the average and the difference was chiefly due to the lessened prevalence of diarrhoeal diseases.

In the years 1904 and 1906 I had as a Medical Officer of Health to deal with two severe epidemics of summer diarrhoea.

During the 1904 epidemic eighty-three deaths from diarrhoea were registered among a residential population estimated at 42,000 giving a diarrhoea zymotic death rate of no less than 1·97. The diarrhoea mortality among 1007 infants born in the registration district during that year reached the very high figure of 67·0. Had a similar rate of mortality prevailed among the infants throughout the year it would have resulted practically in decimation of that portion of the coming race which were being artificially fed.

In 1906 the zymotic death rate from diarrhoea in a community of 50,000 persons was 1·43, and the infantile diarrhoea mortality figure was 55·7.

In the former year (1904) by means of a "spot map" the great majority of the deaths from diarrhoea were shewn to have occurred in streets in proximity to brick fields in which were deposited daily some thirty tons of fresh house refuse. There had resulted an enormous accumulation of hundreds of tons which attracted and bred incalculable numbers of flies. Directly the meteorological or seasonal conditions became favourable for their development.

Close inquiry reveals that "age" incidence in fatal diarrhoea is of supreme importance. Thus, during the two epidemics I have mentioned there were one hundred and fifty deaths from diarrhoea among infants under three years of age, as compared with only five deaths from diar-

rhoea at all other ages, and three of the five at all other ages were persons over sixty-five years of age; whilst no fewer than one hundred and thirty of the one hundred and fifty under three were actually under *one* year of age. I attribute the incidence on the very young and the very old to the fact that milk enters so largely into the dietary at both extremes of life, quite as much as to their enfeebled degree of resistance to disease.

The problems suggested by the facts which I had accumulated were fully discussed in special reports at the time (1904—1906).

I may here be allowed to briefly set out the main conclusions I arrived at as follows:—

(1) Epidemic diarrhoea is essentially *not* a disease of *entirely* breast-fed infants: in other words the tender wholly breast-nourished babe subjected to all such other conditions as heat (or a ground temperature exceeding 56° F. at a depth of four feet), or overcrowding, or organic emanations, &c., will not as a general axiom die of diarrhoea if his food is strictly limited to human milk *from the breast* (see exceptions mentioned on page 157).

(2) There is a close coincidental connection between the prevalence of flies and the prevalence of epidemic diarrhoea.

(3) Dirt is without doubt a most important factor in epidemic diarrhoea. At the same time it is a noteworthy fact that dirty conditions exist in certain localities all the year round, and yet diarrhoea is a distinctly seasonal disease limited to only a few weeks or months in epidemic sense. Therefore there must be some special factors besides the mere presence of dirt or collections of refuse or manure. In this connection we again note the coincidental fly season.

The facts obtained by careful observation, duly recorded, and later the facts adduced by analysis of the results of carefully planned investigations, appear to indicate that the important factors in an epidemiological sense in connection with the prevalence of fatal diarrhoea are at least five in number, viz.

(1) age, (2) foods, (3) meteorological conditions, (4) dirt and (5) prevalence of flies.

Now since the factors of age, food and dirt are constantly in operation all the year round in the neighbourhood, for instance, of refuse deposits, manure heaps, midden privies, stables, &c., it is evident (since epidemic diarrhoea is distinctly a seasonal disease) that in themselves they are incapable apart from the other two factors of giving rise to the disease.

As a matter of fact all five factors in cooperation are necessary.

The meteorological factors have for many years received consideration because fatal epidemic diarrhoea was so marked a seasonal disease occurring in late summer and early autumn.

The conclusions Dr Ballard arrived at from his classical study of the question are well known, and on them he based a provisional hypothesis which held the field as a working hypothesis until, as I have said, I disputed its accuracy at a meeting of Medical Officers of Health at Leytonstone in November 1902. It was again attacked at the meeting of the Epidemiological Society of London in January 1903.

We must not forget however that Ballard himself was careful to call it only a "provisional" hypothesis and moreover that he did not say that "Diarrhoeal mortality was always in evidence when the four-foot earth thermometer reached 56° F." Much scientific research has been carried out since Ballard's time in the hope of discovering a micro-organism as the essential cause of epidemic diarrhoea, but it is more probable that different organisms account for different outbreaks in different localities.

The single verbal modification which in 1903 I suggested in Ballard's provisional hypothesis makes it applicable to the theory which I have advanced elsewhere (May 1906) that the house-fly is the principal agent in epidemic diarrhoea. I regard the ordinary fly, both *Musca domestica* and *Homolomyia*, in its capacity of germ carrier to be "in this sense the essential cause of epidemic diarrhoea." Residing ordinarily in the form of egg, larva, or pupa, either, to use the words of Ballard, "in the superficial layers of the earth," "or" (in some deposit of organic matter such as house refuse or manure) "upon the earth," it there becomes "intimately associated with the life processes of" organisms such as putrefactive moulds and bacteria which are capable of manufacturing "virulent chemical poisons." These poisons, if taken into the human system, are the essential cause of the symptom of intestinal flux or diarrhoea as well as of the severe constitutional symptoms which constitute the syndrome "epidemic" or "zymotic" diarrhoea.

The vital manifestations of the house-fly, as its life-history demonstrates, are "dependent upon conditions of season and on the presence of dead organic matter which is its natural pabulum."

On occasion in its natural life-history when it emerges from the pupa state a winged insect, it is "capable of getting abroad from its primary habitat," and having become air-borne (on its wings) "obtains opportunity for fastening on organic matter" in the shape of man's food, or his excreta or on any refuse, &c.

Some forms of organic matter undergoing fermentation it uses "as nidus" for laying its eggs and thus providing its larvae with appropriate "pabulum." Other forms of organic matter, such as are found in the larder or on the breakfast table, are as acceptable to its insatiable appetite as is also excretal filth, whether on napkins in the nursery, or in the house privy; whether found on the roadside or in the ashbin; and if it has the opportunity, it has no scruples about dividing its attentions equally between the various forms of organic matter whether food or filth which all comes equally acceptable to its voracious maw.

The following axioms will not be gainsaid:—

(α) Fatal epidemic diarrhoea is practically negligible except among infants. It is essentially a disease of infancy. The disease plays great havoc among artificially fed infants only. It is, generally speaking, only a summer disease.

(β) Epidemic diarrhoea is in England as a rule more prevalent in certain provincial industrial centres than in London.

The reasons might be elaborated at some length—but I shall briefly summarise them as follows:—

(α) (1) Artificially fed infants are generally fed on cow's milk or on condensed milk.

(2) Flies are particularly partial to milk, whether fresh or condensed, bearing on their heads, legs, and bodies large numbers of bacteria, generally putrefactive and occasionally "specific" in nature.

(3) Flies are as a rule abundant in hot weather. The more numerous they are the greater in number are the deleterious germs they are capable of conveying to milk, the chief food of the hand-fed infant.

(4) In hot weather the germs introduced by the flies multiply at such a rate that in even two or three hours the bacteria carried by each fly have multiplied exceedingly and have converted the milk into a dangerous poison capable of exciting diarrhoea.

In 1904 (p. 1403) I gave my opinion that the few cases of epidemic diarrhoea which do occur among breast-fed infants could be accounted for by (a) want of cleanliness on the part of the mother, (b) the abominable dummy teats which are allowed to fall on the floor or to be settled on by flies and are then often replaced in the infant's mouth without even a wipe or after only a casual apology for a wipe on a dirty apron, or (c) directly through germ laden flies settling on or even inside the mouths of sleeping infants. This last explanation has received additional confirmation in a paper recently published by Dr Glover (Oct. 1908).

(β) The great provincial industrial cities to a very large extent still have conservancy systems in existence, the privies being infested with flies and their larvae. These privies are now being rapidly replaced by water-closets and the diarrhoea mortality in these cities is lessening *pari passu*.

Old-fashioned privies form both nidus and pabulum for flies, their eggs, and their larvae, as well as for countless billions of putrefactive organisms which sometimes become "specific" pathogenic moulds and bacteria. Being situated within very short distances of houses overcrowded on a small area, with very inadequate curtilage, is it any wonder that flies having to travel but a few feet from the privy to the larder, these cities suffer in larger proportions than London which is a wholly water-closet city, notwithstanding the heavier rainfall which occurs in the north-western districts? Properly constructed, properly used, and properly scavenged earth-closets however do not breed flies, but if improperly used and inefficiently scavenged they are likely to do so.

In Manchester and in New York particularly flies have been definitely traced from building to building. The flies were previously caught and "marked."

(γ) *Municipal Investigations.* The great corporations of Manchester and Liverpool have within the last few years devoted considerable attention to the "fly" question. Indeed such work dates almost from the time of the memorable discussion on Professor Delépine's paper on epidemic diarrhoea early in 1903 to which I have alluded—which indicates that the distinguished Medical Officers of Health of these important cities were not slow to follow up the lines then indicated.

An attempt has been made to throw doubt on the transmission of diarrhoea by flies by the fact that often the diarrhoeal curve has begun to decline, while the number of flies still remain excessive. Dr Niven (1904—05) of Manchester suggested an exhaustion of susceptible material.

Dr Hamer points out that this fails to explain the phenomena in years of very low mortality—but I hold that the amount of diarrhoea depends upon the numerical abundance of flies visiting food and more particularly milk, directly after or within a few hours of settling on objectionable matter whether out of doors or within a dwelling place.

My observation is as mentioned in many of my former papers and reports, that although flies may appear to be quite as numerous in houses after the diarrhoeal curve begins to descend when the tempera-

ture of the air falls perceptibly, they are less active in their habits and do not peregrinate to the same extent, in consequence of the cold, but make for the warm corners of the room, and pay less attention to feeding. Directly the warmth abates flies become sluggish—and a temperature under 45° F. for a few hours renders them quite torpid. From the torpid state they may be restored to activity by removal to a warm room before too many hours have passed; but frost, as I said before, soon kills them.

Dr Hamer thinks that the facts at present ascertained are not such as to enable a positive opinion to be expressed as to the influence of flies in spreading disease in this country. He is apparently largely led to this opinion by the circumstances of an outbreak of typhoid fever which was attributed to flies bred in deposits of house refuse some two hundred or three hundred yards to the S.W. of the affected area, while almost coincidentally an outbreak of dysentery also attributed to the flies occurred at an asylum some five or six hundred yards to the N.W. of the place of the same deposit of house refuse.

I cannot see why Dr Hamer should think that flies can be excluded as active agents in both these outbreaks. It is possible that in neither case were the infecting germs brought from the refuse heap in which the flies were bred. It is equally likely that a typhoid "carrier," that is a person carrying typhoid germs or a convalescent from typhoid fever, had left infective material somewhere in the neighbourhood of the area which was typhoid infected, this infective material being then transferred from one patient to another by the aid of flies. The same argument might easily apply to the outbreak of dysentery in the asylum at the other position. "Dysentery" is a common disease in asylums and probably nearly every asylum has a "carrier" of this disease.

The flies in the asylum precincts then might easily have acted as carriers. But apart from this possible explanation there is nothing improbable in the supposition that at one side of a large refuse heap made up of the refuse of numerous houses there might have been some dysenteric polluted matter, while at the opposite side there might equally have been some typhoid material.

In the absence of any stated evidence as to other possible or more probable sources of these outbreaks it is of course impracticable for one unacquainted with all the facts to judge all the relative probabilities—but one can logically and forcibly maintain that there is considerable ground for assuming in the absence of more definite causal circumstances

that the flies in each district probably were active "carriers" of infection in each instance.

(V) **Literature on Flies in relation to Disease.**

(a) *Earlier Literature.*

The literature on the subject of insects as carriers of disease was fully summed up to date in the year 1899 by Professor G. H. F. Nuttall, F.R.S., Quick Professor of Biology in the University of Cambridge. In his classical work "On the *rôle* of insects, arachnids and myriapods, as carriers in the spread of bacterial and parasitic diseases of man and animals. A critical and historical study," over 350 references are given up to that date. They extend back even to the 18th century, but the majority are references to work and papers during the 19th century, especially the latter part of the last century. This classic work of Nuttall's appears to be the first attempt made to gain a general view of the part played by insects, &c., in infectious diseases.

That anthrax was often spread by the aid of biting flies was suspected generally throughout the 19th century. It is interesting to note that all the hot years (and therefore probably "fly" years) of last century (1803, 1807, 1822, 1826, 1834 and 1874) were so-called anthrax years.

Nuttall in 1897 made a series of experiments on flies (*Musca domestica*) which conclusively proved that flies are able to carry the infection of plague and that they die of the disease.

(b) *Some Recent Literature.*

Recent medical literature abounds with statements bearing testimony to the importance of flies when under certain circumstances acting as carriers of the typhoid germ.

Dr S. Monckton Copeman, F.R.S., with the assistance of Professor Nuttall, F.R.S., is now collecting a *précis* of all recent fly literature which will in due course be published, probably in the form of a report to the Local Government Board. I therefore will not here attempt full references but merely mention a few. Dr Tooth, in a paper on typhoid fever in South Africa (*British Medical Journal*, March 16th, 1901), assigned an important share in the spread of infection to flies which

became a terrible pest and seemed to be peculiarly attracted to enteric patients. This last is an oft repeated observation.

Experiments have demonstrated that house flies which have fed on tubercular sputum may serve as carriers of the tubercle bacillus. The spread of leprosy has also been attributed to biting flies.

Since Nuttall published his monumental work, numerous further experiments have proved the capacity of flies to convey the infection of cholera and of typhoid fever. With regard to the latter disease I noted in 1905 an instance of an outbreak where the available evidence pointed to flies as the carriers of infection and I commented on the evidence afforded of the danger of a case of typhoid fever arising in a privy district during the fly season. Since then Dr Farrar and Dr S. Monckton Copeman, F.R.S., in reports to the Local Government Board, have noted similar instances in other districts, and in 1908 another instance has been noted in Newcastle-on-Tyne by Dr H. Armstrong, Medical Officer of Health of that city.

In a paper read on September 8th, 1908, at Liverpool, Sir James Crichton-Browne quotes Dr D. D. Jackson of New York City, who states that he found as many as 100,000 faecal bacteria on the legs, body and mouth of one fly, and has shewn that in that city there is an exact correspondence between the prevalence of flies and the mortality from diarrhoeal diseases. As stated on a former page, I had previously drawn attention in the year 1903 to the parallelism of the *London* diarrhoea curve with the numerical prevalence of flies. I suggested in 1903 that this diarrhoea curve would be found "to follow the life-history of the common house-fly, which as a rule begins to make its appearance in observable numbers in June, rapidly increasing in numbers during July and the beginning of August. Towards the end of August, though flies are often very numerous, they appear to pay less attention to food and more to reproduction."

In September it is common to have frost which very rapidly kills off flies, those which have found refuge in warm kitchens or bakehouses surviving but in rapidly decreasing numbers all through the winter. Very few flies remain after the end of November until the next warm season comes in.

I think it will be agreed after the evidence I have brought forward that I was not unjustified in 1903 in stating that I gave the so-called "harmless" domestic house-fly the first place as a pathogenic or disease-causing agent during the summer months.

Dr D. D. Jackson of New York in 1907 says, "We are spending

considerable time and money in a war on mosquitoes.....Much more to be feared is the common house-fly. This so-called harmless insect is one of the chief sources of infection which in New York City causes annually about 650 deaths from typhoid fever and about 7000 deaths yearly from other intestinal diseases." In the incidence of various diseases, such as yellow fever, &c., it has been noted that the epidemic influence abates when the cold weather comes. The same applies to diarrhoea and to cholera.

Now why do all these epidemic diseases die down when the frosts come? My reply is:—Because frosts kill mosquitoes and flies which are the main agents in transmitting the germs which cause the diseases. The germs themselves are not necessarily destroyed by cold, though they almost cease to multiply. The main point is that no "carriers" are available.

At the meeting of the Academy of Medicine of Paris held on October 17th, 1905, MM. Chantemesse and Borel made a communication on the spread of cholera by flies in which they agree that the fly hypothesis (which I maintain is a proved theory), explains the arrest of cholera during the cold season when flies die, and also the recrudescence which takes place when flies return in hot weather. It explains the seasonal incidence of epidemic diarrhoea in this country also in the same way.

An interesting fact has been brought to light by Dr Jackson of New York which partly explains why when flies first begin to make their appearance they do not cause so much trouble as later in the season.

Dr Jackson says that examinations which were made of flies "at the beginning of the season directly after hibernation shewed that many of them carried only a few bacteria and moulds and little or no faecal matter. Like examinations made later in the year shewed the presence of numerous animal and vegetable parasites, faecal matter in abundance, large numbers and many kinds of germs, and in some cases individual flies carried as many as 100,000 faecal bacteria on their legs, mouth and body." And again he aptly remarks, "The activity of the house-fly is in proportion to the temperature."

#### (VI) Summary and Methods of Prevention.

In discussing methods of prevention it is of course essential to recognise what it is we wish to prevent.

In one broad comprehensive sentence "our main wish is to prevent the fly contamination of food."

This includes the main hygienic reasons.

We know that flies settle on all kinds of food and therefore we are liable to eat and drink some variety of fly-polluted food, but the effect of fly-pollution is not the same on all classes of food—any more than is every individual fly a source of positive danger. A fly carrying typhoid germs on its legs might leave a few germs on a lump of sugar, or a piece of meat, or bread, and to this extent leave dangerous pollution, but bacteria do not multiply so very rapidly on these varieties of food; moreover such kinds of food as meat are not so attractive to house-flies as is milk or condensed milk or moistened sugar where there is considerable moisture. Again, having shewn previously that flies carry numerous germs, we have next to bear in mind that in those more liquid foods, such as milk, which are rich in fats, proteins and carbohydrates, bacteria find most congenial soil and multiply very rapidly. Further, bacteria can travel rapidly through liquid food such as milk and thus pollute the whole jugful; while, on the other hand, their growth would be slow from the original centre of deposit on solid food such as bread. For these reasons milk, whether cow's milk or condensed, is much more liable than any other variety of food to become grossly polluted, liquid milk being capable of becoming very seriously contaminated if only even one or two flies tumble in. On solid food, a fly deposits only such germs as may be on its feet, and the tip of its proboscis, but flies falling into milk have their bacteria-laden bodies and limbs washed continually in the milk and freely distributed by the fly's attempts to get out; so that one fly laved in milk may transmit more germs than twenty flies settling on solid food, while condensed milk is liable to massive pollution through being only partially liquid. Its semi-solid condition permits of flies settling in crowds all over the surface. But it is yet soft enough to allow the more bristly and probably more bacteria-laden part of the fly's feet to sink in and leave many more bacteria behind. I hope I have made it clear that a single fly falling into milk probably equals a score or more of flies on solid food as regards contamination effects.

Flies then bearing on their bodies numerous bacteria—some comparatively harmless, others of putrefactive nature, and occasionally some which are the causal germs of disease and danger to life—undoubtedly grossly pollute milk; and even in the absence of any known pathogenic or disease bearing germs may by the very excess in

the numbers of putrefactive bacteria they introduce into milk cause such chemical changes to occur in the milk, that the effect is like that of a poisonous mineral drug capable of setting up acute diarrhoea.

Even adults may suffer from such fly-pollution of milk (I have so suffered myself), but infants are most liable to danger, and least able to withstand the poisonous effects of these chemical changes in milk. Moreover, since milk is their staple food (whether fresh or condensed) they receive the poison in larger relative quantities than adults.

One fact is quite clear from all published statistics which are now numerous and together deal with the information obtained through inquiry into many thousands of infantile deaths.

It does not matter where such statistics are published, all point to one conclusion, viz. that the incidence of fatal diarrhoea among wholly breast-fed infants is comparatively rare, and almost a negligible quantity as compared with the frightful mortality among artificially fed infants during the warm months when flies are in excessive prevalence.

I have already expressed the hope that it would soon be made illegal to have fly-breeding accumulations within a certain prescribed distance of any dwelling houses. As for brickfields or other places where decaying or organic matter is deposited in quantity, such businesses should be under strict regulations as to such deposits, and they should moreover be far removed from inhabited houses.

Fortunately the advent of the motor car is diminishing the number of horses in towns, but very strict regulations should be enforced as regards existing stables and mews so that all stable-refuse shall be removed at least twice in the week, especially during the summer months, and the manure receptacle be well cleansed. The bin should have a smooth impervious floor raised above the surface of the ground and the manure should be kept in place by a fine wire gauze. A further closer zinc wire net should surround the first at a distance of two or three inches away. This should prevent flies from ovipositing on the manure.

With regard to the collections of manure for agricultural purposes which very largely consist of stable and cowshed refuse, it seems to me that some improvement might be effected, with advantage to the soil and to agriculture as well as to the public health.

I have had the advantage of discussing this question with a practical farmer, and though I will not make him responsible for fully accepting what I am about to say, the impression I gained was that he was quite in agreement with my views.

Shortly, I think a great mistake is made in leaving large manure heaps lying rotting in fields altogether exposed to the air without any covering of earth. It may be that the manure requires what is termed "ripening," but I do not think the process will be interfered with, but on the contrary helped, by covering the deposit over with two or three inches of good earth. In this way the ammonia, which is at present wasted and causes so much offence to the passer-by when the manure lies uncovered, would be absorbed by the covering of earth and be converted by the bacteria in such soil to nitrates, and thus the all-important nitrogen would be retained which otherwise is lost in the air. Even a covering of two or three inches of earth would largely effect this, and at the same time minimise the risk of such a collection acting as a nidus for breeding flies and assailing the nostrils of sensitive individuals.

As regards house refuse this is an ever present problem, and ever a source of trouble and anxiety to those responsible for the public health, whether town council as the sanitary authority or its administrative officers, Medical Officer of Health, Engineer, or Sanitary Inspector.

I am of opinion that the question of the disposal of house refuse needs revision from the very start, that is from each individual house.

Householders in the open country should each have sufficient curtilage or garden in which to dispose of their own animal and vegetable refuse. Where they have pigs or poultry the problem to a large extent is solved. Where not, the waste should be immediately buried *but not more than three or four inches below the surface of the soil* and in the proximity of growing plants and vegetables.

In towns, or where the mistakes of our forefathers have permitted country cottages to be erected in terraces with insufficient curtilage, some method of public scavenging seems to be almost imperative, unless some public spirited person will provide a field at an accessible distance, not for the dumping of refuse but for its intelligent disposal by each householder.

The sin of overcrowding houses on very small plots is chiefly responsible for the expensive sewerage and water supply schemes which have continually to be provided at great expense and involve an increase in rates so obnoxious to many.

I think the problem in most towns would probably be best met by each householder being provided with two or three small bins, one at least a covered one into which only dry ashes should go and which should always be kept covered; another for waste glass or crockery or iron. This need not be covered. Where animal and vegetable refuse

cannot be buried it should be burned at the house itself—unless the sanitary authority can provide for collection either every day or at least twice in the week.

With small bins and separate bins, one man could easily carry both at one time and thus time and expense of labour be saved. Be this as it may, at any rate it would seem that much trouble might be avoided if each householder were required to do the sorting of his refuse into (1) "coke and ashes," (2) glass bottles, and (3) animal and vegetable refuse; keeping the three classes of refuse distinct. This would simplify the question of the disposal of the refuse of towns, and I believe would prove a saving to the ratepayers in such towns where the houses had sufficient yard space or curtilage to provide accommodation for two or three bins.

The coke and ashes would be welcomed by brickmakers; the animal and vegetable refuse by market gardeners and pigbreeders; while a ready sale would probably be effected for the bottles and bones.

As regards the dust bin on the premises, this should never have anything wet put into it; it should contain nothing but ashes and always be kept covered to keep out rain. If wet tea-leaves, vegetable refuse, &c. are put in the dust bin, it soon becomes offensive, and ferments and forms a breeding place for flies, which we are now aware seek out likely fermenting vegetable refuse for breeding purposes.

Where house refuse is collected, on the present system, a refuse destructor or destructors for the immediate burning of the refuse appears to be the only satisfactory method of disposal in large towns.

When a town is disposing of its own refuse satisfactorily it is still necessary for the Sanitary Authority to see that any brickfields or vegetable gardens in the immediate vicinity are not made the refuse dumping grounds of some other centre of population.

I have touched on the discouragement of stables, mews, &c. in towns, and I think that building bye-laws should include a clause as to the situation of the larder in the house.

This should be a separate room preferably of northern aspect with window opening to the external air, protected from flies by wire-gauze.

The proper sanitation of streets I have previously alluded to.

Finally education is the most important point of all. Truly "My people are destroyed for lack of knowledge" (Hosea iv. 6).

We want the education of future mothers in proper ways of rearing and caring for babies.

We want lady health visitors able and willing to assist in spreading

sanitary knowledge, such as the necessity for cleanliness and for covering over milk-containing vessels. They should be armed also with printed leaflets of advice, caution, and instruction.

We want the dissemination by means of lectures and discussions, or otherwise, of information on subjects connected with natural science which may be of benefit to agriculturists, horticulturists, teachers, students and others, so that our water supplies, our milk supplies, and our food supplies generally, may not run unnecessary risk of pollution either at the original source of production or in the course of what is sometimes a long and eventful journey to the consumer.

In these days when the birth rate of the nation is decreasing at a rate which causes uneasiness in the minds of all who love King and country, it becomes all the more important to conserve infant life.

The plague of English summer diarrhoea kills off every year thousands of flourishing healthy infants. Because of their relation to infantile mortality, exceptional importance attaches to diarrhoeal diseases. At certain seasons and in certain localities it is this factor that determines whether the mortality among young children shall be high or low.

The subject is therefore one of transcendent importance to us as a nation. If we can check epidemic diarrhoea, we shall reduce our infantile mortality to such an extent as to largely compensate for the diminished birth rate, for we learn from the report of the Registrar General for England and Wales that throughout the first year of life diarrhoeal diseases contribute largely to infant mortality.

If, as I have dogmatised for years, and as I hope to convince others, epidemic diarrhoea is chiefly spread by the agency of house-flies, we ought by controlling and preventing the development of flies wherever possible, and by taking every precaution to prevent the access of flies to any article of food (particularly fresh or condensed milk) which is used for the feeding of infants, to effect such a saving of infant life as has not been accomplished by any other single measure.

The grossly polluting effect of flies which has been fully proved will not in any way lessen the responsibility of the cowkeeper and dairyman to prevent contamination of milk at its source. As a matter of fact it only accentuates it, because only too often are flies permitted access to milk in cowsheds or dairies, and these are now additional sources of contamination on which stress should be laid to be guarded against by milk producers and vendors. Their responsibility for the cleanliness of cows, cowsheds, dairies, milkers, and utensils has already been clearly

established. But when they have done all that is necessary or can be expected it is still in the homes of the people themselves that the polluting agencies of dirt and of flies must be chiefly controlled and prevented.

Municipal cleanliness and domestic cleanliness must work hand in hand.

From our knowledge of the habits of some classes it would seem almost an impossibility to ever induce clean ideas and ways among them, but we must nevertheless persist and not be discouraged.

I have great hopes that the scheme of Medical Inspection of School Children which has been inaugurated by Act of Parliament throughout the country, will assist largely in this matter. I have said elsewhere that, "The easiest and surest path of safety for infants during the hot months is natural feeding at the breast." Flies cannot pollute the pure supply direct from nature's fount. I believe the terribly high mortality among illegitimate children is due to the fact that only a very small percentage of such children are naturally fed. Every bottle-fed baby is hedged around by dangers, which can be avoided only by scrupulous care and attention to cleanliness, in everything connected with its food supply.

It is to the infant that fly-borne disease in this country is especially fatal. Our future national interests appear to be largely concerned in how we gain and apply knowledge as to the mischief wrought by what for years was called "the harmless fly."

#### REFERENCES.

- CHANTEMESSE and BOREL (1905). [Report of Meeting of Acad. of Med. of Paris.] *Lancet*, Nov. 4th, p. 318.
- CRICHTON-BROWNE, Sir JAMES (IX. 1908). *Liverpool Sanitation*.
- GLEICHEN and KELLER (1790). *Geschichte der gemeinen Stubenfliege*. Gleichen. *Histoire de la Mouche Commune de nos appartements*. J. C. Keller. (Bound together.)
- GLOVER (x. 1908). *Lancet*. Vol. II.
- GRIFFITH, A. (v. 1908). The Life History of House-flies. *Pub. Health*.
- HAMER, W. H. (1908). Nuisance from Flies. *London County Council Report*.
- HEWITT, C. GORDON (x. 1908). Biology of house-flies in relation to Publ. Health. *Journ. Roy. Inst. Publ. Health*, Oct. 1908, also Monograph on *M. domestica*. *Quart. Journ. of Microsc. Sci.*, Vol. LII. N.S.
- HOWARD, L. O. (1902). *Rept. United States Dept. of Agriculture*.
- JACKSON, D. D. (xii. 1907). *Report to Committee of Merchants' Association*. New York.

- NASH, J. T. C. (1902—07). *Reports to Southend Health Committee.*
- (1903). The seasonal incidence of Typhoid Fever and of Diarrhoea. *Trans. Epidemiol. Soc.*, Vol. xxii. N.S.
- (1903). *Trans. Epidemiol. Soc.*, Vol. xxii. pp. 44—47.
- (1903). *The Hospital*, Jan. 31st and Feb. 7th.
- (1904). *Lancet*, Vol. ii. p. 1403.
- (1904 and 1906). *Special Reports on Epidemic Diarrhoea.*
- (1905). The Waste of Infant Life. *Journ. Roy. San. Inst.*, Vol. xxvi. No. 9, pp. 495—498.
- (v. 1906). The Prevention of Epidemic Diarrhoea. *Practitioner.*
- (1906). *Annual Health Report (Southend-on-Sea).*
- NEWSTEAD, R. (1907). The habits &c. of the common house-fly. *Ann. of Trop. Med. and Parasit.* Univ. of Liverpool, Vol. i. No. 4.
- NIVEN, JAMES (1905). *Report on Health of Manchester during 1904.*
- NUTTALL, G. H. F. (1899). On the Rôle of insects, arachnids and myriapods as carriers in the spread of bacterial and parasitic diseases of man and animals. A critical and historical study. *Johns Hopkins Hosp. Reports*, Vol. viii.
- PACKARD (1874). On the transformation of the common house-fly, &c. *Proc. Boston Soc. of Nat. Hist.*, Vol. xvii. pp. 136—150.

## THE BLEACHING OF FLOUR.

By W. D. HALLIBURTON, M.D., F.R.S.,  
*Professor of Physiology in King's College, London.*

A CASE recently tried before Mr Justice Warrington (The Flour Oxidising Co., Ltd. *v.* J. and R. Hutchinson) has brought to light, what I do not imagine is a matter of common knowledge, namely that large quantities of flour both in this country and abroad are artificially bleached in order it would appear primarily to satisfy the fancy of the public for white bread. The popular taste for the appearance of their food naturally leads the caterers to satisfy it; the yellowness of milk and its richness are usually associated together in the mind of the man in the street consequently it is very difficult now-a-days to purchase milk which has not been artificially coloured.

The similar public taste for whiteness in their bread was met until comparatively recently by keeping the flour after grinding for six to eight weeks; in this time what is technically termed "conditioning" of the flour occurs; the flour becomes whiter, and its baking qualities are simultaneously improved. In 1901, however, a patent was granted to John and Sydney Andrews of Belfast which is now the property of the plaintiffs in the recent action. It cannot be doubted that this patent is of very considerable pecuniary value for it enables the miller to accomplish in a few minutes the whitening that previously occupied a couple of months. The defendants in the action were also millers and it was alleged that they had infringed the plaintiffs' patent by using a similar process which in the case was spoken of as the Alsop process. Both processes have this in common that the flour is bleached by exposing it to the action of peroxide of nitrogen fumes.

The subject has been a matter of previous litigation, the Court of Appeal and the House of Lords having decided that the Andrews'

patent is valid, and a similar result followed the case to which I have alluded. After a hearing which occupied twenty-six days, Mr Justice Warrington pronounced judgment in favour of the plaintiffs.

Into the technical and legal questions involved, and upon which this judgment mainly rests, it is no part of my duty to enter. But any problem which deals with the food of the people raises questions affecting the public health, and it was this side-issue upon which I was asked to give evidence on behalf of the defendants' case, and which I propose to treat in the present article.

I have consistently for many years raised my voice whenever possible against adulterations, and against the use of antiseptics and preservatives in foods, on the ground that they are prejudicial to health especially in producing an increased difficulty of digestion due to the inhibiting action preservatives have on digestive enzymes. The stringent laws of the United States against the employment of adulterants of this order have met with my sympathetic approval, for if they err at all, it is an error on the right side, for they prevent the introduction of even the thin end of the wedge.

I must confess that to the non-legal mind it was a little difficult to see why the defendants in the recent action should have made it a part of their case to show that nitrogen peroxide had a deleterious action on the flour seeing that they were themselves anxious to use what was practically the same method for bleaching. So far as I have been able to unravel the legal subtleties of the case, it appears, however, that if they had been successful in proving this point, they would have established that the specification of the patent was so far incorrect, and therefore the patent itself would have been rendered invalid.

The important question for the hygienist is, however, far removed from the purely commercial aspect of the case, and resolves itself into the enquiry whether treatment with nitrogen peroxide does or does not injure the flour in such a way as to render it injurious to the consumer. If the answer to this question is in the affirmative the employment of this method of artificial ageing ought to be prohibited quite independently of the claims of rival patentees.

The process of natural ageing appears to produce two important changes, which are not disputed. One of these is that the flour becomes whiter, and the second is that its "baking qualities" are enhanced. Whether it produces any difference in the digestibility of the bread does not appear to have been specially investigated; one can, on this point therefore, only say that anything that increases the attractiveness

of a food, will other things being equal provide that psychical stimulus which we know is so important in matters of appetite and digestion.

The upholders of the process of artificial ageing maintain (1) that the flour is whiter than freshly ground flour, (2) that the baking qualities of the flour are equally good or better than those of flour naturally aged, and (3) that any effect on digestibility is negligible.

The first of these three propositions is undisputed.

The second is a matter for the bakers and furnished them with the opportunity of demonstrating that "expert" witnesses in this class of the community are not free from the suspicion that their opinions are influenced by the side which happens to have retained their services, a failing which is not confined to "expert" bakers and millers. Certainly the loaves exhibited did not convey much to the non-experts in court. The differences in the appearance of the loaves are of that unimportant kind the detection of which requires the practised eye.

The third question, the one relating to digestibility and possible chemical alterations produced by the reagent employed, was the one on which my opinion was sought, and I consented to give it as it afforded me another opportunity to enter my protest against the doctrine of an important food stuff.

When I was first asked to give evidence, I was entirely unaware of how extensively artificial bleaching of flour is employed; I fondly considered that at least the staff of life had escaped the attention of the decorative artist; moreover I knew nothing from personal experience of the effect of the bleaching process, and therefore I undertook a few experiments in order to test the matter in dispute. The time afforded me for the purpose was far too short, and I sincerely wished as the case proceeded, and especially when under the stress of cross-examination, that I had had six months to work at it, with nothing else to do, instead of about the same number of days which were already largely occupied with other work. The small number of observations I was able to make confirmed those published in America by chemists who have worked at the question more thoroughly; I cannot see my way at present to undertake any further investigations on the subject, but my observations such as they are appear to me to be worthy of record in a scientific journal. The conclusions I have drawn from my own observations and from those of others are briefly, (1) that the reagent employed comes under the heading of preservatives, and by the inventors this is regarded as an advantage; (2) that the presence of nitrogen peroxide or the products of its action on flour (nitrite reacting material) is distinctly unfavourable

to the activity of digestive enzymes and (3) that the action of the reagent is to produce a change in certain constituents of the flour which impairs their readiness of digestion and therefore their nutritive value.

Admitting all the above, it is only fair to grant on the other side that the total amount of such changes in the flour is small, and that the amount of nitrite left in the flour is only as a rule a few parts per million, and even that small amount is reduced by one half in the process of baking. The diminution in digestibility and nutritive value is therefore not very considerable for a healthy adult with normal digestive capacity. Whether the change is sufficient to be serious for infants or invalids is a question upon which there is at present no evidence, although that possibility should always be borne in mind; may be in such cases the old adage about the last straw may be applicable.

If the process of artificial bleaching sanctioned as it now is by law becomes universal, it should at least be confined within the narrow margin just mentioned. Cases have occurred where over-bleaching has considerably exceeded these limits, and the public do not appear to have any guarantee of the amount of bleaching practised in the preparation of any particular flour. What the miller at present cares most about is the whiteness of the product.

The following brief extract from Mr Justice Warrington's judgment puts the matter in a nutshell.

"Even Dr Halliburton did not go further as a summary of what he considered to be the result, than that the process of treatment by the plaintiffs' invention imposes on the human frame just one more of those extra burdens which the progress of civilisation has from time to time imposed on the human frame. Many of us think that there are many modern improvements, the introduction of motor cars, for example, which impose an extra strain on the human frame, but no one would pretend to say that a patent for the invention of a motor car would not have been a useful invention for that reason. With regard to digestibility it seems to me, it is not a practical objection, and even if it is made out that there is a scientific and theoretical action on the flour which may be said to be deleterious, there is no evidence that there is any practical substantial deleterious result of which I can take account."

That really is the crux of the whole question, and those interested in hygiene will have to see in the future that the scientific objection never becomes practical and substantial, just as some of our public authorities are attempting to minimise the dust, smell, noise and other discomforts that attend the use of motor omnibuses.

In certain of the American States, the use of artificial bleaching of flour has been prohibited in accordance with their law against the employment of even traces of preservative materials. However much we should like to see such an ideal made universal, it is necessary for us as practical men to remember that ideals are not always realisable in older countries, where even more necessary and urgent reforms of the law are difficult to attain.

Passing, however, from generalities, to what after all is the main purport of this paper, we will first consider briefly the results of a scientific kind obtained by others in connection with the subject, and finally the experimental work which I have performed myself.

The most extensive work on the effect of artificial bleaching by peroxide of nitrogen has been carried out by Dr Ladd, Professor of Chemistry, who occupies the position of Food Commissioner in the State of North Dakota, and confirmatory experiments so far as digestibility is concerned have been performed by Dr Shepard, Chemist of the South Dakota Experiment Station. These researches have been published in numerous Government Bulletins, but they are also summarised in recent issues of the *Chemical News*<sup>1</sup>.

Professor Ladd's main conclusions are :

1. That the amount of nitrite reacting material left in the flour varies a good deal ; in over-bleached specimens such as may be obtained from flour left in the separators and so frequently subjected to the action of the fumes, there may be even a xanthoproteic reaction produced.
2. The oil expressed from the flour contains nitrogen and its iodine absorbing value is increased.
3. The digestibility of the starch and gluten as tested by artificial digestion by means of diastase, ptyalin, pepsin and trypsin is diminished.
4. The proportion of amino-nitrogen to protein-nitrogen which diminishes during natural ageing remains unaltered or is increased.

This conclusion has been disputed by chemists both in America and this country, and no doubt it is a difficult point to determine accurately for the change is admittedly a small one. In the state of our present knowledge concerning the reversibility of enzyme action, it is possible that both changes may occur either during the ripening of the grain, or even after the grain has been ground into flour. The smallness of the change in either direction makes this particular factor one of negligible importance from the nutritive standpoint, but if Dr Ladd's statement is correct, it helps to establish the contention that artificial

<sup>1</sup> March 6, 13 and 19, 1909. See also Ladd and Bassett, *J. Biolog. Chem.* vi. 75, 1909.

ageing is a different process from the natural one, and much the same may be said regarding the change in the oil, which apart from the part it plays as a flavouring agent is quantitatively unimportant.

5. No actual chemical change could be detected in the starch.

6. The change in the gluten is partly physical, partly chemical. The gluten washed out from bleached flour is not smooth, but knotty, and its water absorbing power is lessened, so that from the baker's point of view it does not make so good a loaf; this, however, is disputed by rival bakers. It is suggested that the chemical change is of the nature of a diazo-reaction, for nitrogen is evolved on treatment with an acid.

7. The diminution of digestibility is due partly to the presence of nitrous acid or nitrite reacting material acting inhibitingly on digestive enzymes; and partly to the fact that the chemical change produced in the gluten renders it more difficult of solution in digestive juices.

8. The loss of digestibility in the bread made from bleached flour though present is less marked than in the flour itself.

Sufficient of these statements have been proved in American courts of law to render the future use of nitrogen peroxide as a bleaching agent prohibitive in the future in certain States.

Dr Ladd has, however, gone further than this and states that a highly concentrated alcoholic extract of flour which was purposely over-bleached in order to magnify the result was fatal to rabbits, their stomachs showing signs of corrosive poisoning. A fatal result followed a similar experiment with a specimen of commercially bleached flour. Others in America have repeated these experiments on animals but with negative results. Drs Luff and Willcox following minutely the directions of Dr Ladd with specimens of flour bleached in this country have also obtained negative results. One naturally attaches more importance to one positive than to many negative findings, but even admitting that Dr Ladd is correct, the amount of toxic material, seeing that his extracts were enormously concentrated, is so small, that one cannot find fault with Mr Justice Warrington when he regards such experiments as bearing on the theoretical rather than on the "practical and substantial" aspect of the question.

My own investigations have been entirely on the question of digestibility, the experiments being made *in vitro*, not *in vivo*, and I was much surprised at the very great lessening of enzymic action which occurs in the presence of quite minute amounts of nitrites.

The first experiments I performed related not to flour but to starch and protein to which small quantities of sodium nitrite were added.

I selected saliva as a type of a starch-digesting fluid, and gastric juice as a type of a protein-digesting fluid.

Equal quantities of dilute starch paste were placed in a series of tubes, and a small equal amount of saliva added to each. Some tubes had no further addition, but others had added to them small quantities of sodium nitrite in the proportions 1 : 1000, 1 : 2000 and so on down to 1 : 32,000. All were then placed in the incubator at 40° C., and tested from time to time to determine the achromic point, that is the instant when the mixture ceased to give any colouration with iodine. This point was reached in the control tube in 18 minutes; in the tube containing nitrite (1 : 32,000) it was not reached until 33 minutes. The time became still longer in the remainder increasing with the concentration of nitrite.

The protein selected for preliminary experiment was fibrin stained with carmine (Grützner's method); a number of parallel tubes were arranged as before differing only in the amount of nitrite added; the hydrochloric acid of the artificial gastric juice would produce from this some liberation of nitrous acid. The depth of the tint owing to the liberation of the carmine from the fibrin undergoing solution was taken as a measure of digestive activity. In tubes containing one part of nitrite (reckoned as nitrous acid) in 8000 digestion was entirely prevented; a small amount of digestion occurred over night in the tube containing 1 part in 16,000, and a small amount occurred in an hour in the tube containing 1 part in 32,000, but this by colorimetry was found to be only one seventh part of that which had occurred in the same time in the tube containing no nitrite.

Seeing that nitrous acid and its salts produce no known chemical action on starch, their inhibiting action on its digestion by amylolytic enzymes can only at present be explained by their action on the enzyme.

But in the case of protein there are two possibilities, action on the enzyme and action on the substrate (protein).

The question I next set before me was therefore this—Does previous treatment of a protein with a nitrite delay its subsequent digestion, even although there may be no actual nitrite present during the digestion experiment?

I therefore took equal quantities of fibrin stained with carmine as before and placed each in the same volume of 0·1 per cent. hydrochloric acid, a strength of acid which in itself causes no appreciable amount of digestion in many hours. To some of the specimens I added one part of sodium nitrite in 16,000, a strength I had previously found delayed digestion markedly. All of these were allowed to stand at 40° C. over

night; the hydrochloric acid would liberate some free nitrous acid from the nitrite, and so would roughly imitate the treatment which flour undergoes during artificial bleaching.

The next morning, some of the specimens to which nitrite had been added were washed entirely free from the liquid in which they had been standing, and then fresh 0·1 per cent. hydrochloric acid in the same quantity as before was added.

My tubes were now in three sets:

1. Those with nitrite.
2. Those previously treated with nitrite.
3. Those to which no nitrite had been added.

Pepsin was added in equal quantities to each and the whole placed in the incubator at body temperature for one hour. The depth of the red tint of the filtered fluid indicated as before the extent of digestion.

The results were as follows:

In set 1, the amount of digestion was very slight.

In set 2, the amount of digestion was slight, but greater than in set 1.

In set 3, the fibrin had almost entirely dissolved and the liquid was consequently deep red.

The results were so striking that I exhibited the tubes in court.

I further investigated the effect of sodium nitrite on the rennet enzyme, and found that the inhibiting effect on its action almost negligible.

The addition of sodium nitrite even up to 1 part to 4000 of milk did not delay the time of curdling; but if the same addition of nitrite (no means being taken to liberate free nitrous acid) is made to the milk 12 hours previously, the time of curdling was nearly doubled.

I think these experiments show that both factors have to be reckoned with, namely:

- (1) The presence of nitrous acid (even in the comparatively innocuous form of a salt) hinders enzyme action.
- (2) Previous treatment with nitrous acid alters a protein in such a way as to render it less readily susceptible to the solvent action of digestive juices.

Passing now to experiments on flour, I selected at random from the large number of specimens sent to me four samples of unbleached flour, and four of bleached flour, with which to carry out parallel experiments. I knew nothing at the time of the amount of nitrite in the bleached specimens.

I first determined the achromic point during salivary digestion carrying out the experiment in the same way as with starch. The achromic point occurred in the following times :

Sample 1.	22 minutes	{	Average for the unbleached specimens 23½ minutes.
„ 2.	25 „		
„ 3.	21 „		
„ 4.	26 „		
„ 5.	32 „	{	Average for the bleached specimens 35½ minutes.
„ 6.	36 „		
„ 7.	29 „		
„ 8.	45 (?) „		

The rate of digestion is thus distinctly delayed in the bleached samples.

In regard to the digestion of gluten I found considerable difficulty ; for gluten separated from flour is adhesive, intractable and difficult of access to any solvent that tries to penetrate it. The carmine method, which I at first attempted, failed because the dye will not penetrate gluten.

Dr Ladd has employed the following method which strikes me as an ingenious one for overcoming the difficulty ; the bottom of a piece of wide glass tubing is plugged with glass wool, and on the plug is placed the pellet of gluten to be digested. This is immersed in a flask containing the digestive solution, and the time is noted when the gluten disappears.

Not knowing of this method I had recourse to the following :—

The same eight flours were taken as before, gluten was prepared from each and the moisture in a part of each specimen of gluten determined in the usual way.

An amount of moist gluten corresponding in each case to 5 grammes of dry gluten was then submitted to artificial gastric digestion, the conditions being parallel throughout. Sixteen hours later, the undigested residue was caught on a filter which had previously been dried and weighed. The filter *plus* the undigested residue was dried to constant weight, and again weighed ; the weight of the filter was deducted, and the undigested residue was found to be as follows in the eight specimens. The experiment was done in duplicate, and the figures given are the mean numbers.

The comparative indigestibility of the gluten from bleached specimens comes out quite clearly. It will be further noted that the loss of digestibility is not proportional to the amount of nitrite reacting

material present in the flour, the figures for which are given in the last column. These figures I was subsequently supplied with and represent the mean results obtained by two analysts (Dr Hehner and Mr Gordon Salomon). From this one would judge that the main deleterious action is exerted by the nitrous fumes while in contact with the flour, and the diminution of digestibility does not depend on the more or less accidental quantity left behind.

	Undigested residue	Average	Parts of nitrite reckoned as sodium nitrite per million of flour
No. 1.	3·01 grammes		0
" 2.	3·35 "		0
" 3.	3·2 "		0
" 4.	3·42 "		0·1
" 5.	3·52 "		5
" 6.	3·67 "		2·5
" 7.	3·82 "	3·662 grammes	1·9
" 8.	3·64 "		1·3

It will further be noted in the preceding table that one of the unbleached flours contained a trace of nitrite; this apparently was a case of accidental contamination, for the flour came from a factory where the bleaching process was in use. It will be seen that this flour gives the worst result of the four unbleached specimens.

The only other experiment I have performed was a comparison of two flours from the same grist, one of which was freshly milled, and the other was milled three months previously and therefore had undergone natural ageing. In the determination of the achromic point, the differences obtained are within the limits of experimental error, the times being in two samples of the freshly milled flour 18·5 and 17 minutes, and in two samples of the aged flour 18 and 17·5 minutes respectively.

A gluten digestion experiment carried out in the same way as that already described gave the following mean results. The undigested residue from 5 grammes of the gluten from the freshly milled flour amounted to 3·491 grammes; and that from the gluten of the naturally aged flour was 3·482 grammes. The difference here also will be seen to be negligible.

Having no appliances in my laboratory for baking I carried out no experiments on the comparative digestibility of the bread made from different flours. The results obtained by those who have had the opportunity of examining the breads show that the lessening of digestibility of the bread is less marked than it is in the flour. This

appears to be partly due to the reduction of the amount of the nitrite reacting material which occurs during baking, and in reference to the protein (gluten) one can only suggest that the process of baking increases the difficulty of digestion of that substance even in unbleached specimens, so that any difference in digestibility between a loaf made from it and one made from bleached flour would not be so noticeable. It can hardly be doubted that this, which after all is the most important question from the standpoint of the consumer, has had considerable influence with judges in deciding as they have that the objection to artificial bleaching is more or less theoretical. But knowing as we do the possible practical dangers which might ensue were millers allowed a free hand in the use of the very strong reagent they employ, it is necessary that a strict watch should be exercised to keep its use within the limits of safety.

## OBSERVATIONS ON THE EVOLUTION OF IMMUNITY IN DISEASE.

By L. NOON, B.C., F.R.C.S.,  
*John Lucas Walker Student, Cambridge.*

(*From the Lister Institute, and the Pathological Laboratory  
of the University of Cambridge.*)

### CONTENTS.

	PAGE
I. Introduction . . . . .	181
II. General description of the experimental disease . . . . .	183
III. Methods . . . . .	185
IV. The accuracy of the opsonic determinations . . . . .	187
V. Importance of opsonic immunity . . . . .	192
VI. Effect of inoculating normal rabbits with killed cultures of <i>B. pseudotuberculosis</i> . Contrast between subcutaneous and intraperitoneal inoculation . . . . .	193
VII. Infection with living cultures, injected into the peritoneum, or into the blood stream . . . . .	198
VIII. The response elicited by inoculation of sterilised vaccine, in rabbits previously infected with the disease . . . . .	203
IX. Living cultures and sterilised vaccines considered as stimulus- material and their efficiency, from this point of view, com- pared . . . . .	210
X. Causes which tend to prevent efficient autoinoculation in disease .	213
Conclusions . . . . .	216
Bibliography . . . . .	217

### I. INTRODUCTION.

THE following experiments constitute an attempt to follow out in detail the stages by which immunity is established, in the course of a generalised bacterial infection (*Pseudotuberculosis* of rabbits). Two

aspects of immunity are considered, firstly the presence, in the circulating fluids, of specific antibacterial substances, and secondly the power of rapidly producing such substances in answer to the specific stimulus. That is to say, attention is directed, not only to the quantity of specific antibodies present on any day of the disease, but also to the response which the animal can make to various doses of bacterial vaccine. For the immune animal is both more vigorous and more sensitive than the normal, in its reaction to a renewed dose of poison (Wassermann and Citron, 1905). Naturally the facts established with regard to one disease only, cannot be predicated at once of other diseases, in other animals. Still it is hoped that the systematic study of one disease may give some help in coordinating the large but somewhat disjointed mass of clinical observation which is already available.

It is not a new observation that, in the course of the severest bacterial infections, the early days are marked by the absence of any specific reaction on the part of the patient. The general inflammatory reaction is present but no specific antibodies are formed. After a shorter or longer period of delay, more or less immunity is developed. One may therefore distinguish two stages in the course of an infection.

- (a) A period of inertia, as regards the formation of antibodies.
- (b) A period of immunising reactions.

Pneumonia affords a good example of this distinction. J. G. Macdonald (1906), working with Bulloch, showed that, in this disease, the opsonic power of the blood remains low for about a week after the onset. At the end of this period, there occurs a decided rise in the amount of pneumococco-opsonins, which is followed regularly within a few hours, by the crisis. Wolf (1906) obtained similar results. In other diseases the onset of an immunising reaction may be neither so sudden nor so decidedly effective, yet the two stages may be recognised, as in the case of enteric fever. It is well known that the agglutinating power of the serum, which marks one of the specific reactions of the organism to this infection, is generally delayed in its appearance till about the tenth day. Malta fever offers another example of such delay, as in this disease the agglutination reaction is not obtained before the fifth day, and may remain absent till a much later period<sup>1</sup>. Reference may also be made to the streptococcal infection which seems to be a constant feature of scarlet fever. It was shown independently in America and England, by Hektoen (1907) and Banks (1907) that, in

<sup>1</sup> Aldridge (1898), Bert and Lamb (1908), Durham (1898).

the first stages of this disease, the streptococco-opsonic index of the patient was generally low, but that a rise occurred in connection with defervescence, though not before the fourth day. Long continued lack of any immunising reaction has been observed by Wright (1907) in various septicaemic conditions, such as streptococcal endocarditis and Malta fever. The same paper shows how the opsonic immunity produced in response to such infections may be fluctuating and inefficient when it has at last arrived. These results in so far as they relate to Malta fever are confirmed by Bassett-Smith (1907).

In order to examine into the production of these phenomena more closely, experiments were made on the following lines:

The opsonic response following the inoculation of killed cultures into normal rabbits was investigated, and it was found that different tissues possessed different powers of response to these inoculations. Then, the effect of injecting living cultures into normal rabbits was determined, and after this the effect of inoculations of killed cultures into previously infected rabbits. The reaction of the infected rabbits towards inoculations of killed cultures was found to be profoundly altered during the course of the disease. A further examination was then made of the respective activities of living and killed cultures, in exciting a response in rabbits already infected with the disease, and recovering from this infection.

After describing these experiments, the present paper concludes with a discussion of the factors which influence the immunising reactions, which are observed in the course of the experimentally induced disease. The starting point and groundwork of these observations is provided by the work of Sir A. E. Wright and his fellow-workers, to which it is a pleasant duty to acknowledge my indebtedness.

## II. GENERAL DESCRIPTION OF THE EXPERIMENTAL DISEASE.

The infective organism chosen was the *Bacillus pseudotuberculosis rodentium*, which is nearly related to that of plague (*B. pestis*). This bacillus is easily regained in pure culture from the blood of a rabbit or guinea-pig dead of the disease. Its discrete, opaque-white, colonies with crenated margins, are readily recognised; and the absence of contamination, after passage, was confirmed by the sugar reactions.

In the earlier part of the work a strain (designated P) was used which was obtained from the Pasteur Institute, early in 1907; more lately

I have used a strain which was kindly sent me by Dr W. E. Marshall from the Lister Institute in May 1908 (Strain M).

TABLE I.

*Sugar reactions of *B. pseudotuberculosis rodentium*.*

Sugar	Reaction	
	24 hours	48 hours
Lactose	—	—
Glucose	Acid	Acid
Maltose	Slightly acid	Acid
Mannite	Acid	Acid
Dulcite	—	—
Cane sugar	—	—
Inulin	—	—
Sorbitae	—	—

The bacillus grows readily on ordinary agar, and an emulsion of bacilli washed off a 24 hours' agar slope culture, by means of broth or saline solution, formed the infective material for inoculation. A measured fraction of the emulsion obtained from one such culture was inoculated directly into the peritoneal cavity of the experimental animal. In rabbits the gravity of the infection may be determined almost at will, by varying the dose between  $\frac{1}{100}$  of a culture, which dose the large majority of rabbits will survive, and  $\frac{1}{5}$  which constitutes an almost certainly fatal dose. For guinea-pigs  $\frac{1}{100000}$  of a culture was found to be an almost certainly fatal dose, death occurring between the 5th and 14th days. Using larger doses, up to  $\frac{1}{30}$  of a culture, the fatal termination was found to arrive with considerable punctuality on the 6th or 7th day.

The intraperitoneal inoculation is followed by a general peritonitis, with a small quantity of turbid exudate. After two or three days the whole surface of the peritoneum, both visceral and parietal, is found to be studded with minute grey nodules, and the omentum is thickened and infiltrated. At this stage very few bacilli are to be found in the peritoneal fluid, but smears made from the peritoneum or omentum show large numbers of bacilli, for the most part contained within large mononuclear endothelial cells. Very soon the infection spreads, and small abscesses are formed in the spleen and liver. The small grey nodules previously noted on the peritoneal surfaces, tend to become absorbed, but some of them increase in size, and go on to the formation

of abscesses, or caseous masses. All the abdominal glands become affected, and a large mass is often found at the root of the mesentery of the small intestine, composed of glands which have adhered to one another and undergone caseation.

In guinea-pigs the infection usually spreads to the thorax, where the mediastinal glands are found severely infected, and the lungs show numerous miliary abscesses. At death the blood always gave a pure culture of the bacillus.

The immunising reactions called forth by such an infection consist in, (1) an increase of opsonin, (2) the formation of agglutinin, (3) the formation of precipitin. No bactericidal or antitoxic substances can be demonstrated satisfactorily. It is not clear what importance attaches to the presence of agglutinin and precipitin, but it appears that an increase of opsonin is of real and vital significance in combating the disease (Part V, and Fig. 5). Consequently, in studying the fluctuations of the opsonic index, we are observing a manifestation of immunity which is of real importance in determining the fate of an infected animal. Furthermore, as will be shown below, the opsonic index gives much earlier information of an immunising response, on the part of the rabbit, than do observations of agglutination (Figs. 2 to 6, 15, 16, 18). The experience of Leishman and his colleagues of the Royal Army Medical Corps, in studying the effect of various antityphoid vaccines, also show the value of opsonic determinations in giving early information of the progress of immunising reactions<sup>1</sup>. And again the opsonic index has been used with success as a guide to therapeutic inoculations, so that in following its changes one was progressing along a road already partially explored. For all which reasons, in these experiments attention has been chiefly directed to the opsonic immunity.

### III. METHODS.

Estimations of agglutination were carried out macroscopically, in capillary tubes of about 1 mm. bore<sup>2</sup>.

Opsonic estimations were made by Wright's method (1903) with the modifications which have been adopted in his laboratory as the result of much experience, since its first publication. I had the opportunity of working at St Mary's Hospital in the winter of 1907—1908, and became

<sup>1</sup> Leishman (1905, 1908), Harrison (1907).

<sup>2</sup> See Wright (1897), and Wright and Smith (1897).

.well acquainted with the work on which Fleming based his article on the accuracy of the method, and some of the sources of error (1908).

The corpuscular suspension used, was obtained from my own blood. About 5 c.c. of blood was drawn from the finger into about 3 c.c. of a 1·5 per cent. solution of sodium citrate, and well shaken. This suspension was then centrifuged, and the corpuscles, after removal of the supernatant fluid, were shaken again with 3 c.c. of a '85 or 1 per cent. solution of sodium chloride. After renewed centrifugation, and removal of the saline solution, the corpuscular sediment was thoroughly mixed. The whole of this material then afforded a uniform mixture of red corpuscles and leucocytes, which could be used to the very last drop, and sufficed, when necessary, for several dozen estimations.

Human corpuscles were used, because they were found to be more easy to work with than those of the rabbit, which are smaller and more granular, and which also have a rather awkward tendency to stick together in clumps. The human leucocytes were quite active in the presence of rabbit's serum, and gave excellent results. There was a slight tendency to phagocytosis of red blood corpuscles, but not enough to cause any inconvenience.

Guinea-pig's serum on the other hand is a very unfavourable medium for human leucocytes, and in the experiments on these animals, guinea-pig's leucocytes were used. The preparation of a satisfactory suspension of guinea-pig's leucocytes at first presented some difficulty, because the blood begins to clot very quickly. Hence if blood from the ear was allowed to drop into citrate solution many small masses of fibrin had time to form, and to these the leucocytes adhered. The masses of clot and leucocytes, thus formed, were all dragged to the end of the film in the process of spreading, and bad preparations were obtained. The method finally adopted with success was to draw blood from the heart directly into some citrate solution. This can be done quite readily as follows. Into a sterile 1 c.c. syringe are drawn up '75 c.c. of citrate solution. Then, the guinea-pig being held for the moment fairly and squarely on its back, on a holder, the needle of the syringe is plunged directly into the heart, entering just to the left of the sternum in an intercostal space. '25 c.c. of blood are then aspirated from the left ventricle, and the mixed blood and citrate transferred to a small tube, containing a further quantity of citrate solution. This tube is well shaken at once, and the rest of the process of washing the corpuscles is proceeded with exactly as in the case of human corpuscles. Suspensions thus prepared gave excellent results.

The bacilli for the emulsion were removed by means of a platinum loop, from an agar slope culture, about 6 hours old, suspended in saline solution ('85 or 1 per cent.) and thoroughly mixed by the aid of a capillary pipette. This suspension was centrifuged for a minute or two, to precipitate clumps of bacilli, and the upper layers removed to a clean tube and mixed again, to insure uniformity. Emulsions were always prepared from young cultures (4 to 6 hours), as the bacilli are larger, stain better, and do not clump so readily.

The serum was obtained from the ear-vein of the rabbit to be tested. A capillary tube, drawn off to a fine point, forms the most convenient instrument with which to make a puncture. The blood was drawn into glass capsules containing about  $\frac{1}{4}$  c.c.

#### IV. THE ACCURACY OF THE OPSONIC DETERMINATIONS.

The accuracy attainable in a series of opsonic measurements depends not only on the skill and patience of the observer and on the perfection of his methods, but on the species of the animal and microbe used. It is necessary therefore to enquire what degree of accuracy was actually attained in the experiments now before us.

##### 1. Accuracy of a single estimation.

The accuracy of a single estimation is the first point to be considered. Having made one determination of the opsonic ratio between sera *A* and *B*, the question is how near this value is likely to be to the true value. Since the whole argument depends on the observation of changes in the opsonic indices of the experimental animals, it is of vital importance to be able to say, that the deviation of a single estimation from the true value will be *small in comparison with the changes which are to be observed*. In the absence of data for a complete mathematical statement, the following method of dealing with the problem may appear reasonable. In several series of observations the indices of all the pathological or inoculated rabbits were determined in duplicate, the counts, as also throughout the rest of these experiments, being made in ignorance of the source of every preparation. Of 210 such pairs of duplicate observations, only 2 show a divergence great enough to affect the form of the curve, of which each should define a point. Of the other 208 pairs, either member may be taken at will, or the curve may be drawn through the mean points, and those characters of the curves, from which deductions are to be drawn, remain entirely unaltered.

Reference to Figs. 29, 30, 31, 37 to 45, and 50, 51, 52 will render this point clear.

### *2. The variation of the control.*

It was found that normal rabbits showed considerable differences in the opsonic power of their sera. These differences were more or less constant, so that a rabbit which had a low opsonic power on one day, would present the same peculiarity for many weeks. This property was not, however, invariable. The normal count, on which the indices of other sera were to be calculated, was therefore obtained by taking the mean of the counts of three or four normal rabbit sera. In the experiments done at the Lister Institute three normal rabbits were used, in those done in Cambridge four. In each case these standard normal rabbits were chosen, at the beginning of a series of experiments, from amongst a considerable number of normal rabbits. The opsonic powers of the sera of the whole batch were first determined, and the normals for future use were selected as giving a fair mean sample of the batch. Under these conditions it was found that a satisfactory control was established. That is to say, no rabbit, when compared with this control, ever showed accidental variations of anything like the magnitude of those obtained after inoculation or in disease; and on the other hand, experiments could be repeated, and gave constantly the same results. The only exception to this, was in the case of inoculations of diseased animals, where the variable conditions of disease made it extremely difficult to obtain an *exact* repetition of a previous experiment.

Towards the end of these experiments one of the control animals exhibited considerable fluctuations of the index, which roused the suspicion that it had become infected accidentally. Its index then rose and was maintained at a high level for several days, confirming this suspicion (Fig. 27). The animal was therefore sacrificed, and it was found to show the changes typical of a naturally acquired infection. There were nodules in the lymphoid tissue of the vermiciform appendix, and of the expanded lower end of the ileum, two small nodules in the liver, and caseating areas in the portal and mesenteric glands.

### *3. The variation of the emulsion.*

It has been pointed out by Houston (1907) and Wright (1908)<sup>1</sup> that in the case of some microbes, especially the *Meningococcus* and *B. coli*,

<sup>1</sup> Wright, *Practitioner*, May 1908, p. 586.

very different results may be obtained when bloods are tested with different cultures all derived from the same stock. "For in association with the attenuation which these microbes undergo upon artificial media they gradually become less and less resistant to the phagocytic attack of normal blood. A comparatively low index is now obtained where before a very high index was obtained, the result being of course simply due to an increase in the denominator in the fraction:

$$\frac{\text{phagocytic count of the patient's blood}}{\text{phagocytic count of the normal blood}}.$$

This phenomenon is found to occur in connection with the *B. pseudotuberculosis*, as is shown in Figs. 1 to 4. For this experiment two cultures of the bacillus were used, (1) strain M, kept on artificial media, with frequent sub-culture, for seven weeks since isolation from its

Fig. 1. Rab. 10.

Fig. 2. Rab. 6.

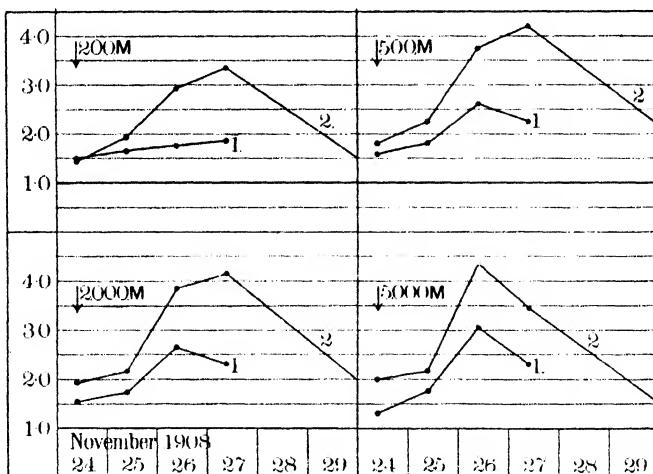


Fig. 3. Rab. 8.

Fig. 4. Rab. 7.

Figs. 1 to 4. Effect of subcutaneous inoculations of vaccine, in rabbits recovering from a previous infection. Influence of attenuation of the culture used in making the opsonic determinations. Curve 1, in each figure, was obtained by using for the estimation of the index a stock which had undergone frequent subculture on agar for 7 weeks; Curve 2, in each figure, by using a stock of the same ancestry, but recently isolated from a rabbit.

Short arrows are used in all figures in this paper to mark the date of inoculations of killed cultures; long arrows denote inoculations of living cultures. The doses are given in millions of bacilli per kilogramme of body weight, or as fractions of a 24 hours' agar slope culture. For the relation between these two measures see Table II.

last passage through a guinea-pig; (2) the same strain which during these weeks had passed through two rabbits, recently isolated from the second. Four rabbits were inoculated with a vaccine made from culture 2. Before inoculation (Nov. 24th), culture 2 gave a higher index than culture 1 in three cases, and a slightly lower index in one case. After inoculation, the indices all rose, but those obtained with culture 2 were in every case higher than those obtained with culture 1. This experiment shows that attenuation of the culture, which is used for the emulsion, may have a great effect on the value of the index obtained. But the process of modification is gradual and progresses in one direction, and so does not interfere with the recognition of changes in the opsonic index induced by inoculations, which changes are more rapid, and have phases both of increase and decrease. This phenomenon of attenuation, however, prevents us from making any trustworthy comparison between the exact heights of the opsonic rises, observed in experiments carried out at different times, or with different strains of the bacillus.

It should be added that the phagocytic counts obtained with pathological sera, giving indices below normal, are increased, during the use of attenuated cultures, in a still greater proportion than the normal counts. Consequently while the normal counts approach those of highly immune sera, low counts approach more nearly to the normal, so that there results a levelling effect. A series of indices estimated with an attenuated culture preserve the order of their relative positions, but all are nearer the normal. It will be seen that this fact provides a further test for the detection of attenuation during a continued series of daily observations, wherever the number of animals under experiment contains individuals with both high and low indices. An accidental variation in the mean of the normal counts, would cause a corresponding alteration of all indices (both high and low) in the opposite direction to this variation. Attenuation of the culture, on the other hand, lowers the high indices and raises the low indices.

The process of attenuation has been described as gradual, but one cause of sudden attenuation has come under notice during this work. When a strain of the bacillus has been grown for some time on one brew of agar, transference to a fresh brew has several times produced a marked attenuation in the first culture on this new and slightly different medium.

4. *The kind and amount of selection exercised in presenting the results.*

In selecting the experiments, here presented, from the whole mass of experimental material collected during about 15 months' work, it was felt necessary to define the principle of what constitutes a fair method of selection, as opposed to an unfair method. If the methods of observation are liable to error, it is obviously possible to obtain, through error, a number of results which favour a preconceived opinion, whilst other results are contrary or doubtful. From such a total of data it is unfair to select those results alone, which favour the conclusion drawn. On the other hand, where all the data obtained favour one conclusion, confirming each other, it is fair to select for presentation a typical series of experiments. Following this principle, attention must here be drawn to certain observations which have been rejected, because they were of doubtful interpretation. The reader will then be able to judge whether the conclusions drawn from the published experiments are materially weakened by these doubtful results.

One curve was rejected because it contained one of the two pairs of widely divergent duplicates mentioned above, the rest of this curve was in harmony with the three given in Figs. 40, 41, and 42, which it confirms. The observations of one day (Feb. 13, 1909) were rejected because of the sudden attenuation of the culture used on this day, as shown by the fact that the indices obtained (7 high and 4 low) showed a marked tendency to approach the normal. Reference to Figs. 11, 12, 15, 16, 17, 46, 47, 48, 49, will show that the indices obtained on the 12th were distributed between 0·35 and 2·2. Those obtained on the 13th fell all between 0·64 and 1·78, ten out of eleven indices having come considerably nearer to the normal<sup>1</sup>. On the 14th a less altered culture of the same strain was used, and six out of seven indices showed again wider deviations from the normal, the seven being distributed between 0·26 and 2·5. It was clear, under these circumstances, that the measurements of the 13th had been made with a different scale to those of the preceding and following days, and they were omitted from the printed curves as obscuring their true form. In addition to the rejections explained above, a considerable number of experiments have been omitted as only serving to confirm points already amply demonstrated in the published figures.

<sup>1</sup> These indices are shown on the charts by small circles.

## V. THE IMPORTANCE OF OPSONIC IMMUNITY.

The general proposition that a state of well-being in the patient of a bacterial infection, is correlated with a raised opsonic index, is supported by a considerable mass of evidence obtained from observations upon man<sup>1</sup>. The following experiment, upon this subject, is given here, not as adding very much to the accumulated evidence of experience, but because it relates to the particular disease under consideration. I hope to be able shortly to carry out further experiments on somewhat similar lines.

Ten guinea-pigs were taken, whose opsonic indices had been estimated on two previous occasions, and found to differ widely from each other. It was thought likely that they would show corresponding differences in their powers of resisting an infection. Such wide differences, in the opsonic indices, are usually found, with regard to this bacillus, in a batch of presumably normal guinea-pigs, in which an autopsy reveals no sign of any previous infection. The disease is so fatal to guinea-pigs, that one can hardly suppose that a considerable percentage of adults have, at some time, acquired a relative immunity by way of the alimentary canal. One would be more inclined to regard these differences as natural or inborn, and comparable to the variations from the normal observed by Wells (1908) in healthy infants; but whichever view be correct matters little to the present argument. These ten guinea-pigs were inoculated on the same day, all with equal doses of the *B. pseudotuberculosis*, given intraperitoneally. The dose used was a small one, namely the one hundred thousandth part of a 24 hours' agar slope culture of the bacillus, isolated two days before from the heart's blood of a guinea-pig, dead of the disease. The animals were left untreated under the same conditions, and the course of the disease was observed. Determinations of the opsonic indices were made from time to time. Within ten days of the date of infection, seven of the guinea-pigs had died. The opsonic histories of all ten were then sorted into three groups, according to the period for which each guinea-pig had survived infection. A different opsonic curve was then seen to be characteristic of each of these groups, the opsonic curves in the same group showing a general agreement in form. A mean curve was therefore constructed for each group, and the three mean curves are presented on Fig. 5.

<sup>1</sup> Macdonald (1906), Wright (1907, 1908), Inman (1908), Hektoen (1907), Banks (1907), Bassett Smith (1907).

It is seen that the three guinea-pigs which lived longest (group I.)<sup>1</sup> had shown high indices previous to infection, and also showed a capability to produce a relatively large increase in opsonin, in response to the stimulus of disease. The animals, on the other hand, which died earlier (groups II. and III.), were those with a past history of medium or low indices, and their indices remained low during the disease.

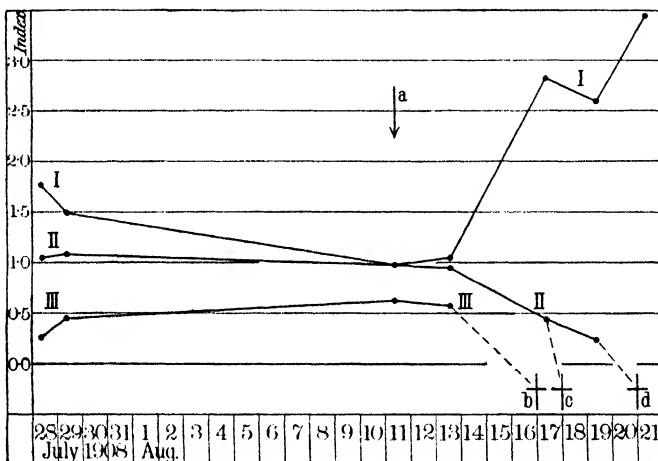


Fig. 5. Correlation between a good opsonic response, and an increased power of resisting infection.

Curve I. Mean opsonic curve for 3 guinea-pigs which survived the longest.

Curve II. Mean opsonic curve for 6 guinea-pigs which survived from 6 to 10 days.

Curve III. Opsonic index of 1 guinea-pig which only survived 5 days.

a. Date on which all the guinea-pigs were infected intraperitoneally, each with 100,000 of a culture.

b. One guinea-pig died.

c. Four died.

d. Two died.

## VI. EFFECT OF INOCULATING NORMAL RABBITS WITH KILLED CULTURES OF *B. PSEUDOTUBERCULOSIS*; CONTRAST BETWEEN SUBCUTANEOUS AND INTRAPERITONEAL INOCULATION.

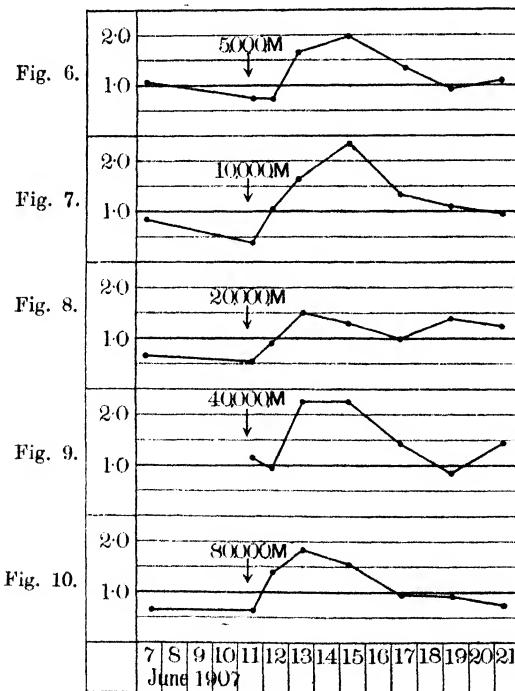
### 1. Subcutaneous inoculation.

The material for inoculation consisted of an emulsion of bacilli in 85 or 1 per cent. sodium chloride solution. The bacilli were washed off the

<sup>1</sup> Of these three guinea-pigs two died within a fortnight of infection, one recovered, and was killed by accident after 6 months. No traces of the infection were discovered at the autopsy.

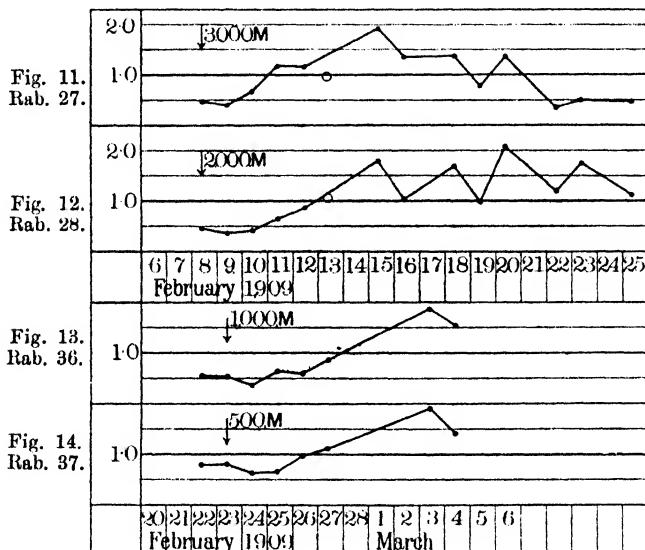
surface of a 24 hours' agar culture with the saline fluid, and, after enumeration of the bacilli against the red corpuscles of normal blood, by the method of Wright, the emulsion was heated to 60° C. for half an hour or an hour. Doses were estimated numerically, as so many millions of bacilli per kilogramme of body weight of the inoculated rabbit. For convenience this is denoted by the abbreviation M. which is used, in citing doses, to mean *millions of bacilli per kilogramme of body weight*. Doses were usually made up to the volume of about 1 c.c. for subcutaneous inoculation. Some very big doses had, however, a larger volume.

A series of normal rabbits received subcutaneous inoculations ranging from 160 M. to 80,000 M. Doses from 5000 M. up to the largest given, all produced the same response, as measured by the *rapidity and duration* of the rise in the opsonic index, which followed the inoculation (Figs. 6 to 10). Reference to these figures will show that the general



Figs. 6 to 10. Response of normal rabbits to subcutaneous inoculation of large doses of vaccine.

form of the curve is the same in all five cases, the differences between the curves being probably within the limits of error at this early period of the work. This form of response has been regarded as the typical form for subcutaneous inoculation, and the *minimum excitatory dose* (5000 M.), necessary to call forth this immediate reaction in a normal animal, is referred to in what follows as the M.E.D. (subcutaneous). Various doses, given in disease, or given intraperitoneally, are compared to it as to a standard. Doses smaller than 5000 M. produced a response which was delayed for some days, and did not as a rule reach so high a maximum (Figs. 11 to 14); delay was the most marked feature, characteristic of the response to small doses, in contrast to the immediate effect produced by the larger doses.

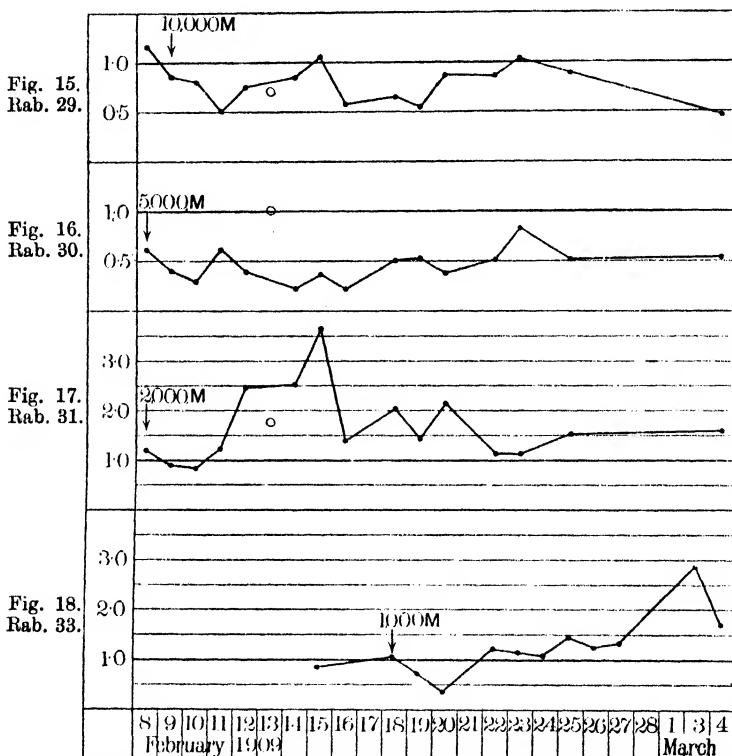


Figs. 11 to 14. Response of normal rabbits to subcutaneous inoculation of smaller doses of vaccine.

## 2. Intraperitoneal inoculation of normal rabbits.

When the vaccine is given intraperitoneally the response evoked is quite different from that obtained by subcutaneous inoculation. The most striking feature observed is a preliminary fall of the opsonic index (Figs. 15 to 18), a fall which is more marked and more prolonged the larger the dose used. The most favourable dose seems to be one of about 2000 M., for the rabbit which received this dose showed a very

marked rise, following an initial fall of the index, which lasted for two or three days (Fig. 17). This contrast between the effects of subcutaneous and intraperitoneal inoculations, is a strong argument in favour of the local production of antibodies<sup>1</sup>. It appears, moreover, that the *B. pseudotuberculosis* is a microbe peculiarly well adapted for the exhibition of the differences of reaction, which are due to inoculation at different sites. This may be explained, in all



Figs. 15 to 18. Response of normal rabbits to intraperitoneal inoculation of vaccine.

probability, by supposing that the bacillus produces only a very limited amount of soluble toxic matter, so that the effects of injection of a vaccine are limited more strictly to the site of inoculation, than in the case of many other microorganisms. For it has been found to be true in most cases that subcutaneous injection of an antigen is followed

<sup>1</sup> Wassermann and Takaki (1898), P. Romer (1901), v. Dungern (1903), Wassermann and Citron (1905).

in the first instance by a decrease in the quantity of the corresponding antibody circulating in the body fluids. This was first shown by Brieger and Ehrlich (1892), who estimated the antitoxin content of the milk of a goat, which was inoculated with tetanus toxin. Salomonsen and Madsen (1897) confirmed these observations, by estimations carried out on the blood and milk of a mare, inoculated with diphtheria toxin. Wright (1901, 1903) showed that similar effects followed the inoculation of antityphoid vaccine; his observations referring to the bactericidal power of the blood serum. Wright (1907) quotes similar results again, with respect to the opsonic and agglutinating powers of the blood of men and animals, inoculated with various bacterial vaccines. Further, it has been shown that, in some cases, during this period of low antitropic power, the injected antigen may be detected in the circulating blood (von Dungern 1903). Under these conditions we cannot hope to observe the pure effect of a local inoculation, as it may be masked by the diffusion of the inoculated material through the system. Conversely, in a case where inoculations in different situations produce widely different effects, we may surmise that these effects are chiefly due to a localised action. In the case of the *B. pseudotuberculosis*, its low toxicity provides another argument for the very small amount of its soluble poisonous products; large numbers of living bacilli have to be present in order to kill an infected rabbit, and extraordinarily large doses of vaccine have to be used in order to produce any effect on a normal rabbit. This is clear when one remembers that the prophylactic dose of *B. typhosus* for a man contains about a thousand million bacilli, and very few therapeutic doses as large as this have been used in respect to any of the bacterial infections of man, which have been inoculated with success. While therefore a thousand million of the bacilli, with which we are familiar, usually constitute an exceptionally large dose for a man, a normal rabbit weighing 2 kilogrammes must receive subcutaneously ten times this number of *B. pseudotuberculosis* before the typical response is produced. Parallel with this low degree of activity exists a considerable difficulty in producing a low opsonic index even in a diseased animal by subcutaneous inoculation with this bacillus (Part VIII. p. 203), which seems, therefore, to form very little noxious material in a soluble form.

From the above it will be seen, that the effect of subcutaneous inoculation of pseudotubercle vaccine differs both from that of intraperitoneal inoculation of the same vaccine, and from that of subcutaneous inoculations of soluble toxins, or of vaccines which contain much soluble

antigen. The most probable reason for this is, that we are here studying the effect of a purely local response of the subcutaneous tissues at the site of inoculation. On the other hand, when the vaccine is given intraperitoneally, the very rapid interchange of lymph between the peritoneal cavity and the general circulation, gives every opportunity for the absorption of the circulating opsonin, even if the vaccine material is not itself washed into the blood stream. If this be granted it will follow that the subcutaneous tissues are specially adapted to perform a protective rôle, against bacterial invasion. This rôle they fulfil by reason of their peculiar ability to react rapidly to a sufficient stimulus, and to dispose, in some way, of an excess of bacterial matter, without allowing the general stock of circulating opsonin to be diverted in order to combine with this surplus. It remains for future investigations to determine how far this speciality is confined to the subcutaneous tissues. The comparison here is only between these and the peritoneal system, by which term is denoted the peritoneal cavity and the extensive lymphatic areas, which are in easy connection with it. It is possible in view of the success which has attended the method of immunisation by way of the gut, that the mucous or submucous tissues may possess powers similar to those of the subcutaneous tissues. The experiments of Leishman (1908), however, with typhoid vaccine weigh against this surmise. It should also be pointed out that, when immunity has been established, other tissues acquire this property of rapid response, as will be shown in a later part of this paper<sup>1</sup>.

## VII. INFECTION WITH LIVING CULTURES, INJECTED INTO THE PERITONEUM OR INTO THE BLOOD STREAM.

If we introduce a living emulsion into the peritoneal cavity the series of events which follows this infection, is again different from either of the cases treated in the preceding part. If the rabbit is to survive for more than a day or two, the dose must not exceed  $\frac{1}{5}$  of a 24 hours' agar slope culture, which quantity contains about 6,000,000,000 bacilli, of which nearly all are living (see Table II).

The injection of such a dose as  $\frac{1}{5}$  of a culture is followed by a considerable fall of the opsonic index, which is maintained for several days (Figs. 19, 20, 21). It has been shown in Part VI. that such a fall may be

<sup>1</sup> Compare also Wassermann (1905), *Deutsch. med. Wochenschr.* p. 1101.

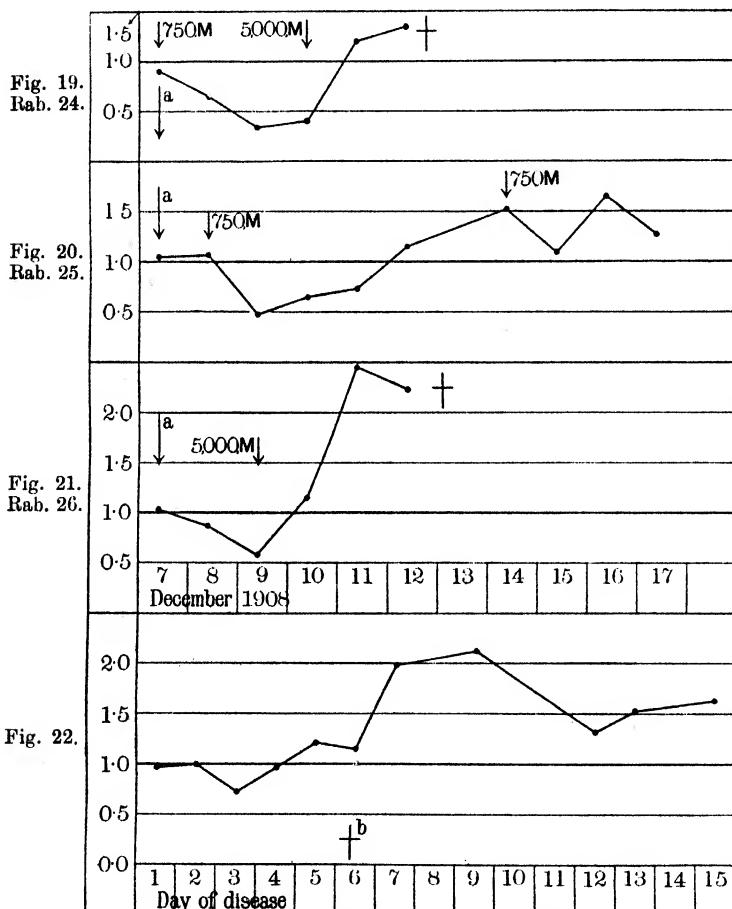
produced by intraperitoneal injection of killed bacilli (Figs. 15, 16, 17, 18), but cannot be produced by subcutaneous inoculation. The living bacilli are, therefore, just as well able to abstract opsonin from the circulating fluids, as those killed by heat. On the other hand, even with their power of multiplying and spreading into the substance of the abdominal organs, they do not form any more efficient stimulus to the defensive mechanism of the animal. The production of increased quantities of opsonin is delayed just as much in the case of the living inoculation, as it was in the case of the dead vaccine. When a smaller quantity of living microbes are used for the inoculation, that is a quantity less than the minimal efficient intraperitoneal dose of bacilli killed by heat, there is an additional period of delay, while the infecting microbes have time to reach the minimal number efficient as a stimulus. This is illustrated by the experiment shown on Figs. 32 to 36 where the dose inoculated was  $\frac{1}{100}$  of a culture, or about equivalent to an inoculation of 150 M.; also by Fig. 19, which represents the mean of 9 curves, obtained in experiments where the infecting doses ranged from  $\frac{1}{100}$  to  $\frac{1}{4}$  of a culture. From these observations it would appear that, though the bacilli have early invaded and occupied in force the omentum, liver, spleen, and abdominal lymphatic glands, and though they are even circulating in the blood stream, yet they have not, at this time, gained access to any tissue which has the power (presented by the subcutaneous tissues) of a prompt and vigorous immunising response.

TABLE II.

Number of culture tubes	Average number of microbes per tube, enumerated against red blood corpuscles	Number of living microbes by culture
4	28,000,000,000	—
1	31,000,000,000	26,000,000,000
1	31,000,000,000	31,000,000,000

After an inert period of shorter or longer duration, during which the opsonic index remains low, a reaction at length sets in, and the index rises to a high level which is only maintained, however, for a few days (Fig. 22). After this the opsonic index shows a tendency to fall gradually, though further autoinoculations occur from time to time, inducing reactions which often surpass that first produced, in intensity. An example of this was seen in the case of rabbits 19, 20, 21 and 22, whose indices rose during the first reaction to little over 2 (Figs. 32 to 36), whilst a fortnight later they were found to have attained an average value of about four (Figs. 39 to 42; Jan. 1 and 2).

Of the rabbits infected with moderate doses (less than  $\frac{1}{2}$  of a culture), four died before the first reaction set in (Figs. 22 and 36), three on the sixth day, and one on the tenth day of the disease; whilst all those which survived the inert period, and produced an immunising response, as shown by the opsonic index, went on to ultimate recovery; none died. Of those which received  $\frac{1}{5}$  or  $\frac{1}{4}$  of a culture, on the other



Figs. 19 to 22. Response of normal rabbits to intraperitoneal inoculation of living cultures.

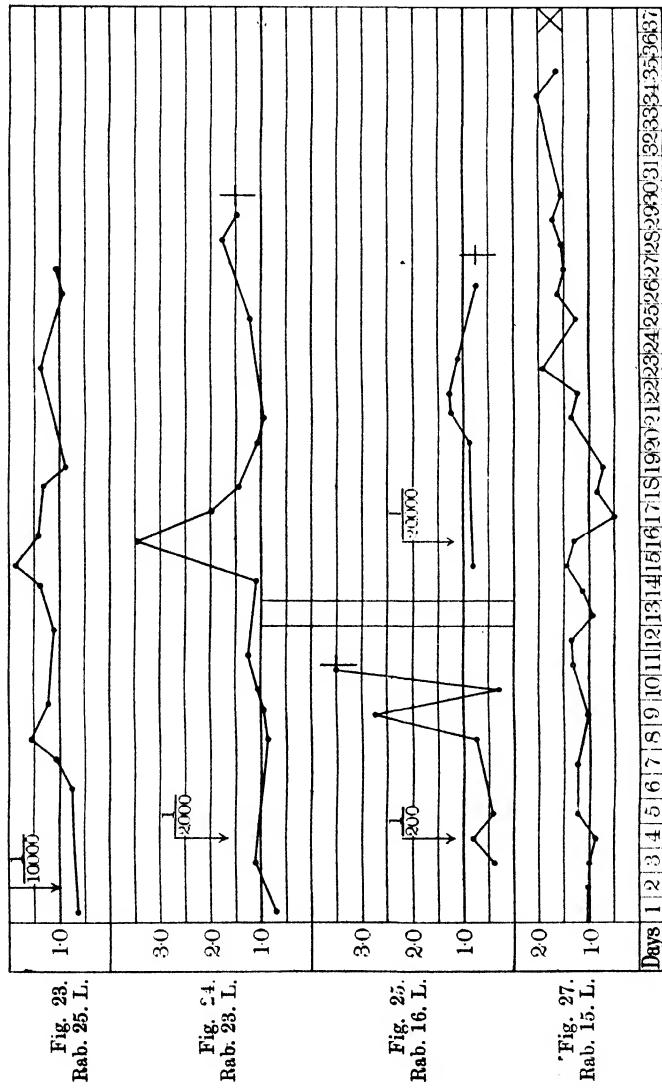
Figs. 19 to 21 show also the effect of various subsequent subcutaneous inoculations.

Fig. 22. Composite curve of opsonic indices of 9 rabbits which were infected with living bacilli on day 1.

+ signifies the death of the animal.

a. Three out of the 9 rabbits died.

hand, three out of four died (Figs. 19, 20, 21), in spite of an immunising response, elicited in two cases by the subcutaneous injection of vaccine and in the third case supervening in the ordinary course of the disease



Figs. 23 to 26. Response of normal rabbits to intravenous inoculation of living cultures.

Fig. 27. Variations occurring in the opsonic index of a rabbit during the development of a naturally acquired infection. The exact date of the infection is not known.

× The animal was killed, and the diagnosis confirmed.

(rabbit 25, Fig. 20, died on the 26th day of the disease). It was, of course, only to be expected that a massive infection would be able to overcome the protective mechanism of the animal, even when excited to its utmost activity.

Intravenous inoculation of living cultures produces a more fatal disease than intraperitoneal inoculation. No rabbit survived infection by way of the blood stream with more than  $\frac{1}{10000}$  of a culture. Some of the rabbits which received doses between  $\frac{1}{20000}$  and  $\frac{1}{200}$  of a culture lived long enough to produce an immunising response. In these the inert period was at least as long as that following infection by way of the peritoneum, but it was not accompanied by any marked decrease in the quantity of circulating opsonin (Figs. 26 to 29). The bacilli therefore do not multiply in the blood stream to any great extent (as may be also shown by blood-cultures), but they become lodged in various tissues throughout the body, and are shut off from the circulation, within the necrotic foci or abscesses thus formed, so that the amount of opsonin abstracted from the circulating blood remains comparatively insignificant. The immunising reaction, when it appears, is markedly fitful and ill sustained.

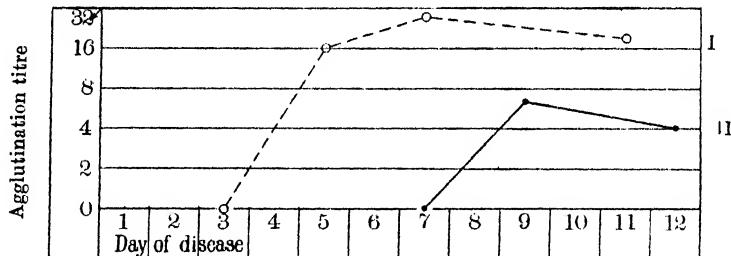


Fig. 28. Interrupted line; development of agglutinin in five rabbits which received subcutaneous inoculations of vaccine on day 1.

Continuous line; development of agglutinin in three rabbits which received intraperitoneal inoculations of living cultures on day 1. The figures at the left-hand side denote the dilutions of the serum, in which a distinct agglutinating reaction was obtained.

Fig. 27 shows the changes in the opsonic index, which occurred during the development of an infection, acquired accidentally by way of the alimentary canal.

#### *Agglutination experiments.*

The absence of any immunising reaction during the first period of the disease, which has been shown above by reference to opsonic

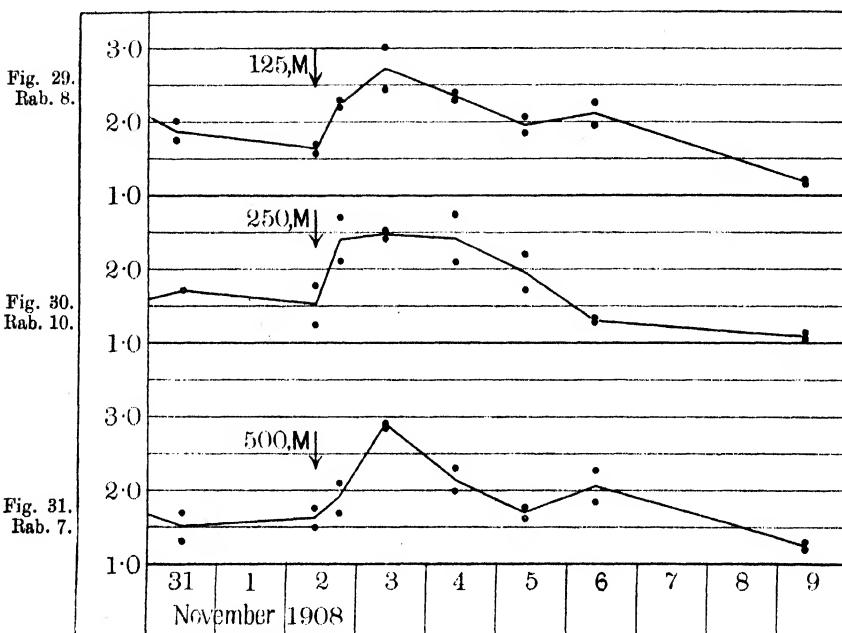
observations on a series of 23 rabbits, may be equally easily demonstrated by agglutination experiments. Three infected rabbits, on which these experiments were made, only produced agglutinin after the lapse of eight days from the day of infection (Fig. 28; Curve II.). On the other hand, subcutaneous inoculation of five different normal rabbits, with various doses of killed bacilli, led to the discovery of a relatively high agglutination titre, in every case, after only four days from inoculation (Fig. 28; Curve I.).

### VIII. THE RESPONSE ELICITED BY INOCULATION OF STERILISED VACCINE, IN RABBITS PREVIOUSLY INFECTED WITH THE DISEASE.

During the first stage of the disease caused by inoculating a living culture into the peritoneum, the experimental animal still reacts to a subcutaneous inoculation of vaccine in the same manner as a normal rabbit does. This fact enables us to state with more certainty the cause of the decrease in the opsonic content of the blood, which is observed during this period. This decrease was attributed solely to the abstraction of opsonin from the circulating fluids by the infecting bacilli. But there were two further possibilities, namely that the production of opsonin had been inhibited, and the supply thus cut off at the source, or, on the other hand, there might be an increased production of opsonin, which was hidden from observation because masked by the rapid absorption of this from the blood, by the bacilli. These possibilities are however disposed of by the fact mentioned above, which was established by the following experiment.

The three rabbits, 24, 25, 26, were inoculated, each with  $\frac{1}{5}$  of a 12 hours' agar slope culture, on the same day. After 48 hours, rabbit 26 was obviously weaker than the other two, and appeared likely to die within the next 24 hours. It seemed that, if the presence of a severe infection can ever inhibit or mask the production of opsonins, this rabbit was in a condition to exhibit the phenomenon. It was therefore given a subcutaneous injection of 5000 M.—the M.E.D. for a normal animal. During the next two days the opsonic index rose to over 2·4 (Fig. 21). On the next day, rabbit 24, being at the time, as far as one could judge, *moribund*, received a similar subcutaneous inoculation. It lived long enough to show a rise of the opsonic index up to 1·34 (Fig. 19). It is clear that an appropriate stimulus, even in this stage of the disease, can excite the production of opsonin in increased quantity; an increase which the presence of a very grave infection is unable either to inhibit

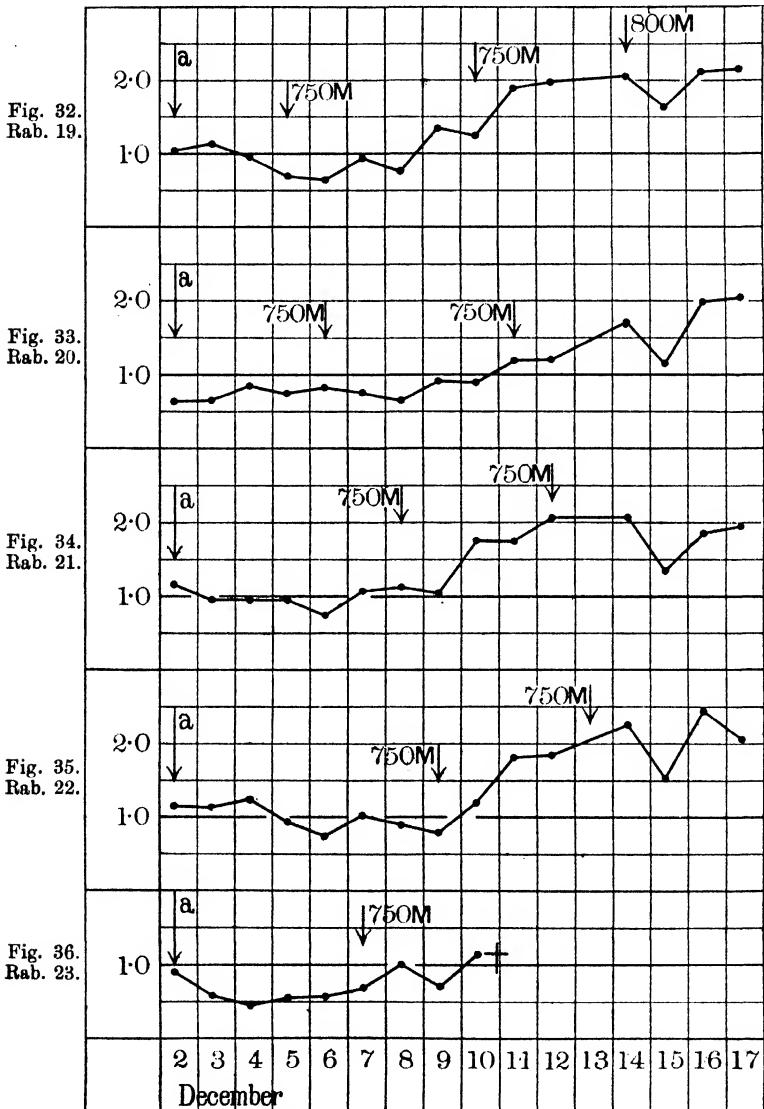
or to mask. The lowered index, which marks the beginning of this intraperitoneal infection, is therefore due only to the absorption of opsonin by the bacilli introduced; it is accompanied neither by inhibition, nor by any efficient stimulation of the immunising mechanism.



Figs. 29 to 31. Response of previously infected rabbits to small doses of vaccine, given subcutaneously.

After a certain degree of immunity has been attained in the course of the disease, the reactions of the animal towards injections of vaccine are entirely altered. In the first place it becomes possible to elicit a response with a dose one hundredfold less than the M.E.D., and secondly it becomes possible to cause a temporary lowering of the opsonic index, by subcutaneous inoculation of suitable doses of vaccine. The tissues of the (relatively) immune animal have acquired a new property, by virtue of which they react with increased vigour to the presence of microbes of the species which has induced the immunity. This tissue-immunity, which outlasts the presence of an increased quantity of antibodies in the body fluids, has been emphasised by Wassermann and Citron (1905).

The response to small doses was first obtained in three rabbits (Figs. 29, 30, 31), one of which received the inoculation on the 15th day of the disease (rabbit 10, Fig. 30). An experiment was therefore



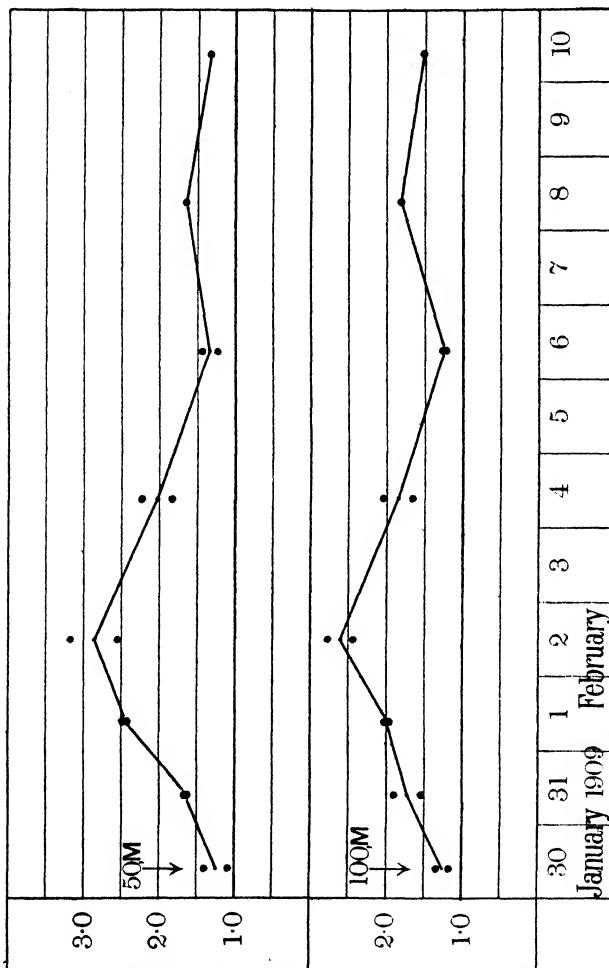
Figs. 32 to 36. Opsonic charts of rabbits which were infected at (a) with doses of  $\frac{1}{10}$  of a culture. Subsequent inoculations of moderate doses of vaccine, subacute, produced no immediate response.

instituted to determine when this property of sensibility to small doses first appeared. It was obvious that if test inoculations were made, the opsonic indices of the inoculated animals should give an indication of the effect, or absence of effect, of inoculation, within 24 hours.

For this experiment 5 rabbits were infected intraperitoneally on the same day with equal doses ( $\frac{1}{10}$  of a culture) of the *B. pseudotuberculosis*. While their opsonic indices were estimated daily, they were given test doses of vaccine at intervals. The dose of vaccine chosen was 750 M. that is, 15 times the minimal effective dose for a sensitive immune rabbit, and rather less than  $\frac{1}{4}$  of the M.E.D. (Figs. 32 to 36). Reference to the figures will show that certainly none of the inoculations before the eighth day were effective. Rabbit 22 (Fig. 35) gave a rise of index following immediately on an inoculation on the eighth day, but, at this time, one expected a rise in the ordinary course of the disease, so that it remains uncertain whether this dose had any effect or no. Similarly inoculations on the 9th and 12th days (rabbits 19 and 22; Figs. 32 and 35) had a doubtful effect, while doses on the 10th and 11th days (rabbits 20 and 21; Figs. 33 and 34) had no effect. In rabbit 10, however, as mentioned above, a dose of 250 M. given on the 15th day of the disease, had produced an undoubted response (Fig. 30). This rabbit had received a larger infecting dose, namely  $\frac{1}{10}$  of a culture. The conclusion is, that the condition of increased sensibility to inoculation is only established some days later in the course of the disease than a high opsonic index.

The response of a diseased and sensitive animal to a small dose may have one of two forms. Either there follows immediately on the inoculation a very rapid rise of the opsonic index, which is already well begun within the first six hours, and which reaches its maximum in about 24 hours (Figs. 29, 30, 31), or there is a gradual rise, which begins slowly and goes on with increasing rapidity to a maximum after 48 to 72 hours. Such a response as this last has only been seen (in these experiments) in animals which had already received several doses of vaccine (Figs. 1, 2, 3, 4, 37, 38). The first form is that more familiar to those who have treated, by inoculation, various bacterial infections in man. Passing on to examine the effect of larger doses, it is found that the response elicited is irregular, and shows periods of decrease as well as of increase in the opsonic index (Figs. 39 to 45). The response in disease, therefore, differs from that of a normal rabbit, in the fact that a "negative phase" can be produced, which may or may not be preceded by a temporary rise of short duration, whereas in normal rabbits,

subcutaneous injections of pseudotubercle vaccine are not followed by any negative phase (Figs. 6 to 14). The dose required to produce this effect in a diseased rabbit varies in different rabbits and in different stages of the disease, and is also influenced by the height of the opsonic index at the time of inoculation, and by previous inoculations. Thus doses of 500 M. (Fig. 45) and 410 M. (Fig. 42) were both sufficient to cause a temporary fall of the index, in rabbits not previously inoculated



Figs. 37 and 38. Response of rabbits, recovering from a previous infection, to small subcutaneous doses of vaccine.

with an effective dose, whereas the inoculations of Nov. 24th (Figs. 1 to 4), ranging from 200 M. to 5000 M., all alike acted as small doses, that is produced a pure rise of the index in rabbits which had each been inoculated on several previous occasions. It may be pointed out that Figs. 32 and 45 refer to one rabbit, the interval elapsing between the two inoculations being five weeks, during which time 2 intervening inoculations were given. Similarly Figs. 30 and 46 refer to one rabbit; there was the same time interval, of five weeks, and 3 intervening inoculations.

The inoculations shown on Figs. 39 to 42, illustrate the ease with which an already high index is lowered by inoculation, of even a relatively small dose. The four rabbits used for this experiment all had indices before inoculation of about 4. The doses given on this occasion ranged from 410 M. to 50,000 M. and the response evoked was the same in every case, the most marked feature being a very rapid fall of the index during the first 24 hours succeeding inoculation.

With regard to this experiment it should be stated that the persistence of a lowered index was partly due to the alteration of the strain from which the emulsion was prepared each day. Thus the culture used on Jan. 1st was only the third subculture since isolation of the bacillus from a rabbit's blood. But as subcultures were made once, and sometimes twice a day, that used on Jan. 6th was considerably attenuated. This attenuation being suspected on Jan. 7th, a return was made to the second subculture since isolation, and a slope was sown from this. The indices obtained, with an emulsion of this culture, were all a good deal higher than those for Jan. 6th. There is, however, no doubt that the first effect of the inoculations of Jan. 2nd, was a very pronounced fall of the opsonic index of each rabbit; and this fall was produced in one case (rabbit 22, Fig. 42) by a dose less than one tenth the M.E.D.

To sum up the foregoing section: an animal which has a raised sensibility with regard to inoculation of vaccine, reacts differently according to whether small or large doses are given. Small doses produce a pure rise in the opsonic index, large doses produce a temporary fall; but the dividing line between what constitutes a "large dose," and what a "small dose," varies considerably, and depends on the condition of the animal.

With regard to the response of a diseased animal to inoculation, the question arises whether the dose administered may not suffice to induce an inflammatory reaction at the foci of disease, just as tuberculin or

Fig. 40. Rab. 20.

Fig. 42. Rab. 22.

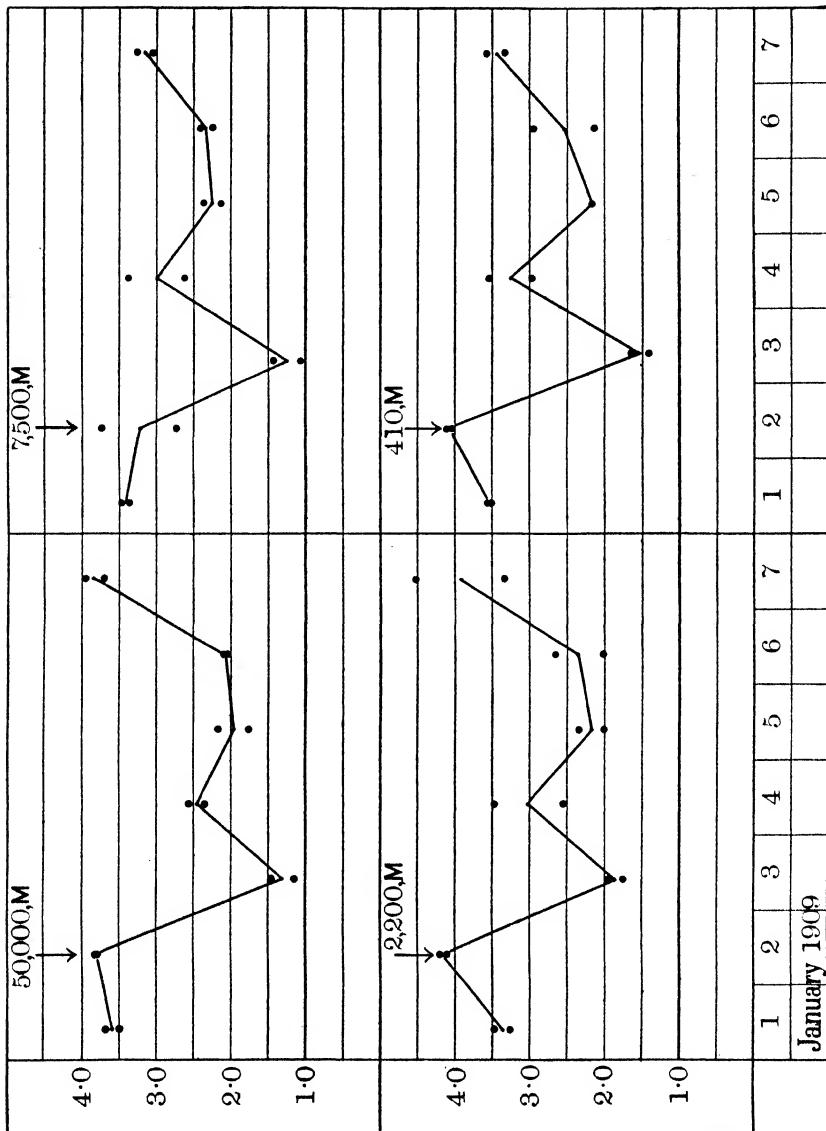


Fig. 39. Rab. 19.

Fig. 41. Rab. 21.

Figs. 39 to 42. Response of rabbits, previously infected, to large and moderate subcutaneous inoculations of vaccine. The index, which was very high in each case before inoculation, falls immediately.

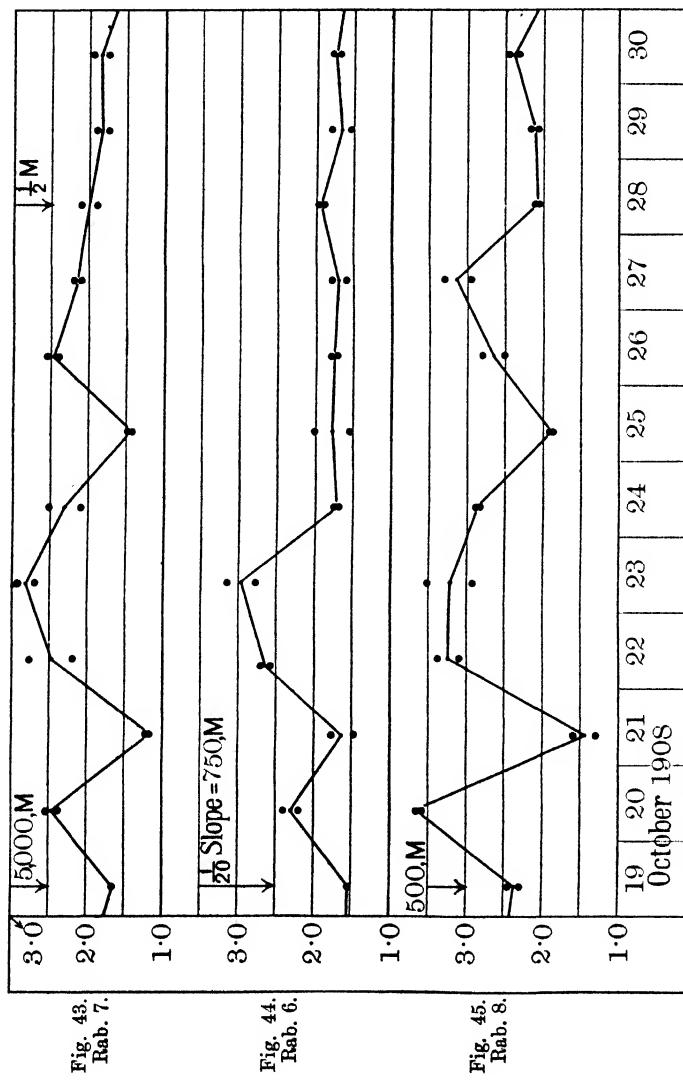
mallein may, when used in veterinary practice as a test for tuberculous disease or glanders. Such a reaction, by increasing the circulation, would tend to carry the bacterial products more widely afield, and in larger quantity, and thus a secondary autoinoculation could conceivably follow as a consequence of an inoculation from without. For the present, however, we want sufficient data for the elucidation of this complex question.

#### IX. LIVING CULTURES AND STERILISED VACCINES CONSIDERED AS STIMULUS-MATERIAL, AND THEIR EFFICIENCY, FROM THIS POINT OF VIEW, COMPARED.

The question whether the antigen, which excites the production of opsonin, is a metabolic product of the growth of the bacillus, or, on the contrary, a product of its disintegration, is of no little interest from the point of view of vaccine therapy. If the first alternative were true, the inoculation of a living culture would provide not only a maximum quantity of ready-formed antigen, but also a source of continuous supply of fresh antigen. But if the second alternative be true, then it is possible that the process of sterilising the vaccine may increase its immediate value as stimulus-material, by breaking up the bacilli, and rendering them more readily available to the tissue cells. In this manner one could imagine a killed culture providing a more intense immediate stimulus, than a similar quantity of a living culture. To test this it was necessary to compare the immediate stimulus-value of bacilli, living, and killed by heat. In making this comparison one can only judge by the immediate results, because the later effects of inoculation would be influenced by the multiplication of the living bacilli within the body. Hence no conclusions can be drawn from the response following intraperitoneal injection, of killed and living cultures, into normal animals. In both cases the response is delayed, and multiplication of the living bacilli has had time to occur before it sets in. The effect of inoculating living bacilli into the subcutaneous tissues might be profoundly influenced by the gross changes accompanying abscess formation, so that this method of comparison between the living and dead would give untrustworthy results.

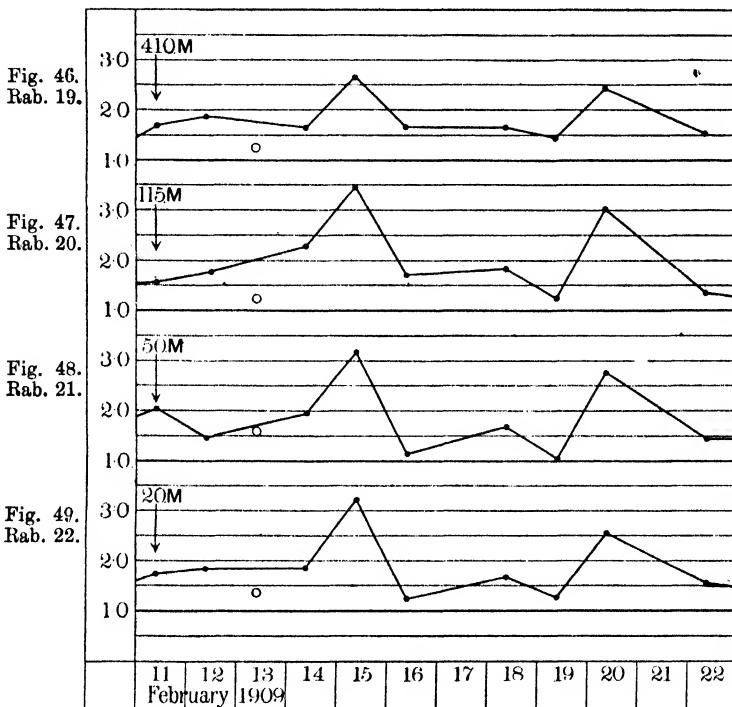
The immune animal, however, can react immediately to intraperitoneal inoculations of either living or dead cultures, so that a fair comparison could be made of their stimulus-value, by making injections into the peritoneal cavities of rabbits which had recovered from a

previous infection, and were consequently in a condition of immunity. A suitable quantity of living bacilli, given in this manner, was found to excite a response similar to that called forth by the injection of killed vaccine, as will be seen by comparing Fig. 44 with Figs. 43 and 45. This form of response was obtained with widely different doses of vaccine (500 M. and 5000 M.), so that the experiment did not give exact information as to what was the vaccine-equivalent of the quantity



Figs. 43 to 45. Intraperitoneal inoculation of living bacilli (Fig. 44) compared with subcutaneous inoculation of vaccine (Figs. 43 and 45). The rabbits on which these inoculations were made were recovering from a previous intraperitoneal infection with living cultures.

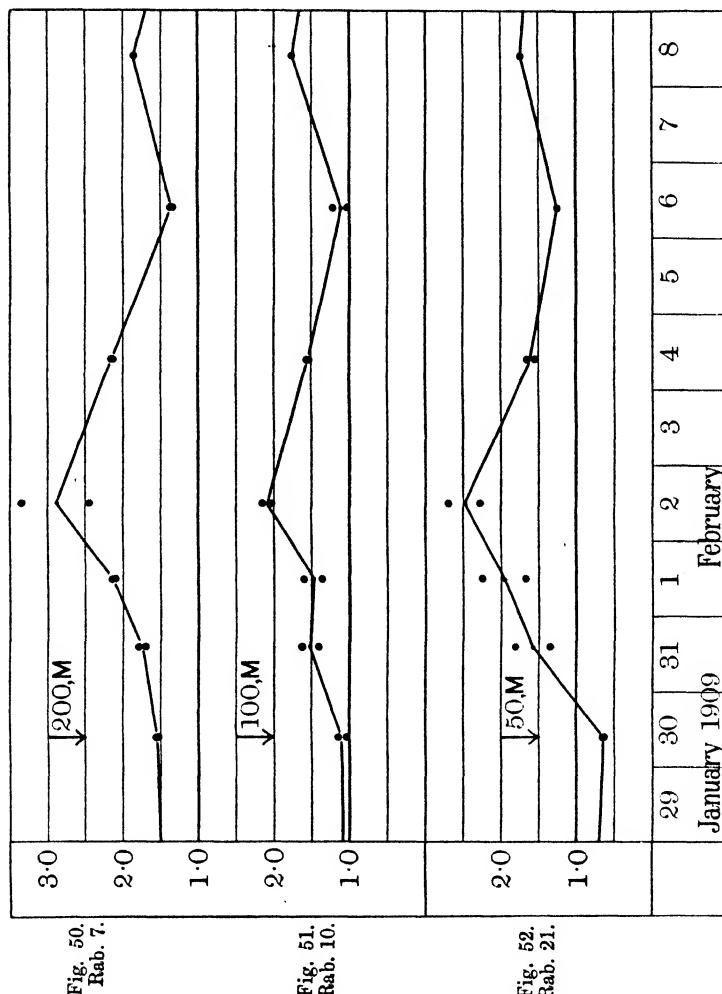
of living culture used ( $\frac{1}{20}$  of a 24 hours' agar slope culture = 750 M.). A second experiment, made with smaller doses, gave more information. Four rabbits received respectively 20 M., 50 M., 115 M. and 410 M. (living culture); none of these rabbits gave an immediate response (Figs. 46 to 49), their indices remained steady for 3 days after inoculation, and only rose on the fourth day. These doses of living bacilli were, therefore, too small to excite at once an immunising response.



Figs. 46 to 49. Response of rabbits, recovering from a previous infection, to intraperitoneal inoculations of living cultures.

In contrast to this series, three rabbits were given respectively 50 M., 100 M. and 200 M. sterilised vaccine. They all gave a response, which began with a slight rise of the opsonic index on the first day, and reached its maximum on the third day after inoculation (Figs. 50 to 52). This shows that a dose of vaccine as small as 50 M. contains more immediately available antigen, than does the comparatively large dose (410 M.) of living bacilli. The living bacilli on the other hand, by the decay of successive generations, keep up a constant supply of antigen,

which ultimately produces a corresponding reaction on the part of the animal. It will be observed that this reaction followed the smallest dose, as well as the largest dose given.



Figs. 50 to 52. Response of rabbits, recovering from a previous infection, to intraperitoneal inoculations of vaccine.

#### X. CAUSES WHICH TEND TO PREVENT EFFICIENT AUTOINOCULATION IN DISEASE.

The first desideratum, for an efficient reaction, is that it should be prompt. But it has been shown that one tissue differs from another very markedly in the power of prompt response to inoculation, the

subcutaneous tissue possessing this power in a high degree, whilst it is absent from the tissues of the peritoneal area. Hence an autoinoculation can only be considered efficient, which affects tissues gifted with this ability to react immediately; and hence also much of the antigen set free within the body will be wasted on tissues of low reactive power. The locality affected by the disease thus becomes of supreme importance, especially in the first stage, before an immunising response has been set up. After the infected animal has acquired a certain degree of immunity, other tissues have been educated to respond readily to the stimulus of inoculation, so that efficient autoinoculation is more readily attained, than it was at the beginning of the disease. But it still remains a matter of chance coincidence if the liberated antigen reaches the tissue best able to react to its presence.

The opsonic charts of rabbits infected by the blood-stream, so far as they go, entirely support this argument. Figs. 23 to 26 refer to rabbits, which suffered from severe generalised infections. In spite of the gravity of the infection, the opsonic charts (Figs. 23, 24, 26) show evidence of the occurrence of remarkably few effective autoinoculations. Rabbit 24. L. indeed, received no effective stimulus at all, in the course of its disease. Rabbit 23. L., during a disease lasting 26 days, only received two autoinoculations; and these were equivalent (judging by the results) to the quantities of vaccine treated of in Part VIII. as "small doses" (Figs. 29, 30, 31). The first, peaked elevation of the opsonic curve, culminating on May 29th, is a typical and active "small dose" response, undisturbed by any succeeding autoinoculation, so that it shows admirably the gradual fall to normal. The second elevation, immediately preceding death, was probably similar in character, but the observations for two days are lacking. Rabbit 25. L. (Fig. 23) also showed a curve indicating small autoinoculations at fairly wide intervals.

About the events of the days intervening between the elevated portions of the curves, one can only deduce that the tissues are receiving a very small amount of bacterial matter. The insignificance of this amount of constant leakage from the foci of infection, is to be argued from its lack of effect as shown on the opsonic charts. But, if it be urged against this view, that we are ignorant of the effect of a constant and diffused supply of bacterial products, bathing the tissues, as it were, in a poisonous medium, then there are two considerations to be met. First, the experiment of inoculating an animal with a large intra-peritoneal dose of bacilli, living or dead, shows that the effect of such a bath, applied to only a portion of the whole tissues of the body, is a

prompt and marked lowering of the opsonic index (Figs. 15, 16, 19, 20, 21), and consequently such a condition, occurring in disease, should give evidence of its existence upon the opsonic chart. Secondly, if we assume that all the tissues have a constant large supply of bacterial matter to deal with, it is difficult to explain how they can respond so readily, as they certainly do, to the additional stimulus of a small dose of vaccine. It follows that the immunising mechanism of these rabbits only received small and intermittent stimuli from autoinoculation, yet the disease, which could only provide so slight a stimulus, was fatal to two of the three, which presented, post-mortem, convincing indications of a grave septicaemia.

The histological relation of the infecting microbes to the tissues is also of importance, since one condition of stimulation is a close contact between the tissue cell and the bacterial matter. Now most of the bacilli, in this infection, are either enclosed in phagocytes, or shut up in necrotic foci, which later become small abscesses. With regard to the first category, we are ignorant whether a phagocyte can produce specific antibodies, and, if it can, whether these are passed at all into the circulation, or are devoted entirely to the enclosed bacilli, which have excited their formation. With regard to the second category, those bacilli, namely, which are growing within a necrotic area, or in an abscess, consideration will show that they are, for a time, practically shut off from the living tissues of the body. When a bacterial embolus lodges, it can be seen, after a few hours, to be surrounded by a zone of dead cells. As the bacteria multiply and grow into this necrotic mass, the necrosis spreads too, so that the advancing colony is preceded in every direction by this zone of death. Except at the first lodgment of the embolus, therefore, no living tissue has contact with the invaders, only a certain amount of their soluble products diffuses out into living areas. And this, for a time, is the only material available as a stimulus. Later, when the affected area has become limited by an active abscess wall, the contents of which have been liquefied, the conditions are probably much more favourable.

It has been shown, however, by Freeman and others (1907), in the case of localised infections, that their presence may fail to call forth any notable immunising response, until the conditions are altered by massage, active or passive movement, or by induced hyperaemia. These measures insure a sudden disturbance of the infective focus, and may be supposed to bring the infecting bacteria or their products into relation with healthy, or at least active tissues, in suddenly increased

quantity. The effect is, undoubtedly, a lively immunising response, which was absent before.

The state of the antigen itself has to be considered, as well as the tissue with which it comes into relation. When a dose of vaccine is given, the antigen is brought into contact, with the tissue elements, all at once, and in considerable concentration. In disease, on the contrary, the antigen is formed gradually by the growth and decay of bacilli spread widely through the animal body. *Pari passu*, with this growth and decay, goes on the absorption of bacteriotropic substances from the circulating fluids, so that each freshly formed bacillus, as well as the products of its growth and decay, is at once partially saturated with antibodies. Such partially saturated antigen constitutes a very much less efficient stimulus to the tissues, than unsaturated antigen, such as that contained in a sterilised vaccine. On this point the experiments of Jorgensen and Madsen (1902) are very clear. These observers found that rabbits and goats, passively immunised by the injection of anti-typhoid sera, would not react to an inoculation of killed typhoid bacilli. But as soon as the passive immunity had passed off, that is, as soon as the agglutination titre of the blood had fallen again to the normal value, the animals would react to inoculations of killed typhoid bacilli, with an energetic production of agglutinin. They argue, from these observations, that, in the case of the passively immunised animals, the inoculated material combined at once with the available antibody, and consequently its stimulating activity became neutralised.

From the arguments set forth above we may perhaps conclude that the causes, which tend to prevent efficient autoinoculation in disease, are the following:—1. The tissues chiefly affected are normally unable to make a prompt specific response to the stimulus which the presence of bacterial matter supplies, such a power being special to the sub-cutaneous tissues. 2. The gradual increase, by growth, of the infecting microbes and their products, which is met, at each step, by a partial saturation with antibodies supplied from the circulating fluids. 3. The temporary shutting up of every large aggregation of bacteria behind a zone of necrotic tissue. 4. (Possibly),—the inclusion of otherwise available stimulus-matter, within the various orders of phagocytes.

#### CONCLUSIONS.

1. Opsonic immunity is of real importance in determining recovery from an infection with *B. pseudotuberculosis*.

2. The subcutaneous tissue of the rabbit has a special power, not possessed by the peritoneum, of reacting promptly to inoculations of killed cultures of this bacillus, with an increased production of opsonin.

3. The above observation is an instance of the local production of antibodies.

4. Intraperitoneal inoculation of *B. pseudotuberculosis*, living or dead, produces an immunising response after a considerable delay.

5. Intravenous inoculation of the living bacillus, produces an immunising response after a considerable delay.

6. Rabbits previously infected with the bacillus are profoundly altered as regards their reactions towards renewed inoculations of living or killed cultures.

7. Cultures of *B. pseudotuberculosis* killed by heat at 60° C. contain more immediately available antigen, than do equal quantities of living cultures, and hence constitute a more efficient stimulus.

8. The following causes tend to prevent the occurrence of efficient autoinoculation in disease:—The tissues affected may not be reactive; the antigen formed within the body is partially saturated with antibody *pari passu* with its formation; foci of infection are largely shut off from active tissues by necrosis; phagocytosis may prevent the ingested bacilli from functioning as a stimulus.

## BIBLIOGRAPHY.

- ALDRIDGE, A. R. (1898). Note on the serum reaction of Mediterranean fever, and its treatment by antitoxic plasma. *Lancet*, Vol. I. p. 1394.
- BANKS, A. J. (1907). The variations in scarlet fever of the opsonic power of streptococci. *Journ. of Pathol. and Bacteriol.*, Vol. XII. p. 113.
- BASSETT-SMITH (1907). The treatment of Mediterranean fever by means of vaccines, with illustrative cases. *Journ. of Hygiene*, Vol. VII. p. 115.
- BIRT, C., and LAMB, J. (1898). Mediterranean or Malta fever, with special reference to the specific agglutinating substances which make their appearance in the blood in the course of that disease. *Lancet*, Vol. II. p. 701.
- BRIEGER and EHRLICH (1892). Beiträge zur Kenntniss der Milch immunisirter Thiere. *Zeitschr. f. Hygiene*, Vol. XIII. p. 336.
- VON DUNGERN (1903). Die Antikörper. pp. 81 *et seq.*
- DURHAM, H. E. (1899). Some observations on the *Micrococcus melitensis* (of Bruce). *Journ. of Pathol. and Bacteriol.*, Vol. V. p. 377.
- FLEMING, A. (1908). Some observations on the opsonic index, with special reference to the accuracy of the method and to some of the sources of error. *Practitioner*, Vol. LXXX. p. 607.

- HARRISON, W. S. (1907). Report on the results of experiments in connection with antityphoid vaccine. *Journ. of the Roy. Army Med. Corps*, Vol. ix. p. 10.
- HEKTOEN, L. (1907). The opsonic index in certain acute infectious diseases. *Centralbl. f. Bakter.*, 1. Abt. Vol. XLIV. p. 456.
- (1907). Is scarlet fever a streptococcus disease? *Journ. American Med. Assoc.*, Vol. XLVIII. p. 1158.
- HOUSTON, T., and RANKIN, J. C. (1907). The opsonic and agglutinative power of blood serum in cerebrospinal fever. *Brit. Med. Journ.*, Vol. II. p. 1414.
- JORGENSEN, A., and MADSEN, T. (1902). The fate of typhoid and cholera agglutinins during active and passive immunisation. *Festschrift ved Indvielsen af Statens Serum Institut, Kopenhagen*, No. vi. pp. 21 and 22.
- LEISHMAN, W. B. (1905). Report on the blood changes following typhoid inoculation. Antityphoid Inoculation Committee (*Wyman and Sons, London*).
- LEISHMAN, W. B., HARRISON, W. S., GRATTAN, H. W., and ARCHIBALD, R. G. (1908). Report on further experiments in connection with antityphoid inoculation. *Journ. of the Roy. Army Med. Corps*, Vol. x. p. 583.
- LEISHMAN, W. B., HARRISON, W. S., GRATTAN, H. W., WEBB, A. L. A., and KENEDY, T. C. (1908). Third report on experiments in connection with antityphoid vaccine. *Journ. of the Roy. Army Med. Corps*, Vol. xi. p. 327.
- MACDONALD, J. G. (1907). Opsonic content of the serum in the course of acute pneumonia. *Trans. Pathol. Soc.*, Vol. LVII. p. 45.
- SALOMONSEN and MADSEN (1897). Recherches sur la marche de l'immunisation active contre la diphtérie. *Ann. de l'Inst. Pasteur*, Vol. XI. p. 315.
- SMALLMAN, A. B. (1909). Preliminary note on the use of antityphoid vaccine in the treatment of enteric fever. *Journ. of the Roy. Army Med. Corps*, Vol. XII. p. 136.
- WASSERMANN, A., and CITRON, J. (1905). Ueber die Bildungsstätten der Typhusimmunkörper; ein Beitrag zur Frage der lokalen Immunität der Gewebe. *Zeitschr. f. Hygiene*, Vol. L. p. 331.
- (1905). Die lokale Immunität der Gewebe und ihre praktische Wichtigkeit. *Deutsch. med. Wochenschr.*, Vol. XXXI. p. 1101.
- WELLS, J. H. (1908). Observations on the opsonic index in infants. *Practitioner*, Vol. LXXX. p. 635.
- WOLF, H. E. (1906). Observations on the opsonic index and the antipneumococcal power of the blood in pneumonia. *Journ. of Infect. Dis.*, Vol. III. p. 731.
- WRIGHT, A. E. (1897). Note on the technique of serum diagnosis of acute specific fevers. *Brit. Med. Journ.*, Vol. I. p. 139.
- (1901). On the changes effected by antityphoid inoculation in the bactericidal power of the blood; with remarks on the probable significance of these changes. *Lancet*, Vol. II. p. 715.
- (1903). A lecture on therapeutic inoculations of bacterial vaccines, and their practical exploitation in the treatment of disease. *Brit. Med. Journ.*, Vol. I. p. 1069.
- (1904). A short treatise on antityphoid inoculation (*A. Constable, Westminster*).
- (1908). On some points in connection with vaccine-therapy and therapeutic immunisation generally. *Practitioner*, Vol. LXXX. p. 565.

- WRIGHT, A. E. (1908). Ueber die Prinzipien der Vaccintherapie. *Gesellschaft deutscher Naturforscher und Ärzte Verhandlungen*. Leipzig.
- WRIGHT, A. E., and DOUGLAS, S. R. (1903). An experimental investigation of the rôle of the blood-fluids in connection with phagocytosis. *Proceedings of the Royal Society, London*, Vol. LXXII. p. 357.
- (1904). Further observations on the rôle of the blood-fluids in connection with phagocytosis. *Proceedings of the Royal Society, London*, Vol. LXXXIII. p. 128.
- WRIGHT, A. E., DOUGLAS, S. R., FREEMAN, J., WELLS, J. H., and FLEMING, A. (1907). Studies in connection with therapeutic immunisation. *Lancet*, Vol. II. p. 1217.
- WRIGHT, A. E., and SMITH, F. (1897). On the application of the serum test to the differential diagnosis of typhoid and Malta fever. *Lancet*, Vol. I. p. 656.

## ON THE ABSENCE OF RESPIRATORY DISORDERS IN THOSE INHALING STARCH DUST OVER LONG PERIODS.

By W. PERO KAUFMANN, M.B.

*Cardinal, Ontario.*

IT is generally conceded that there is no one circumstance associated with occupation which has a more baneful influence upon health than the protracted inhalation of dust.

The resulting morbid conditions of the respiratory tract which ensue are described under the generic heading "Pneumoconiosis," and contingent upon the class of "dust," the workers exposed to its inhalation exhibit lesions differing clinically in their course and intensity according to the *intrinsic* physical characteristics and specific chemical and physiological properties of the particular "dust." We must also bear in mind the factors which act *extrinsically* of the dust: what we may call occupation habits or concomitants, such as, for instance, intemperance, exposure, quality of ventilation, etc.

While these physical, chemical and physiological properties merge one into the other, and render the classification of dusts under these headings difficult, if not impossible, yet we may group them broadly as follows, in consideration of the immediate reactions between the inhaled particles and the tissues and fluids of the respiratory system. Along these lines we recognise :

### 1. *Insoluble dusts.*

Organized : Hairy (wool), Fibrous (cotton, flax).

Amorphous: Angular and sharp, becoming less so in the order stated :—

1. Metallic, calcitic and siliceous particles.
2. Coke and coal dust.
3. Branny particles—(woody and siliceous).
4. Woody particles—sawdusts, etc. etc.

2. *Soluble dusts* : Dusts originated by the grinding of:—

- Nitrate of potash.
- Carbonate of soda.
- Caustic soda, etc.

3. *Dusts not directly soluble* as such, but becoming more or less so through the reaction with the tissues and body fluids.

- (a) Starch granules, largely admixed with protein (12·0 per cent.), and cellular matters (0·3 per cent.), as commercial flour.
- (b) Starch granules substantially freed from protein (0·4 per cent.), and cellular matters (0·05 per cent.), as commercial starch (maize).

The two last classes and a comparison of their effects afford the subject of this paper. In dealing with the former, reference will be made to the work of others, and comparisons sought with the data which I have been able to accumulate regarding the latter class, from observations extending for the last three years (since May, 1906).

Before entering "in medias res," it will be well, I think, to give a brief summary of the process of winning starch, and in so doing, to explain beforehand allusions I shall have to make to the workers in the various departments.

The grain (maize), after being soaked in sulphur water (dilute  $H_2SO_4$ ) kept circulating through the "steeps" for about 72 hours at about 120° F. is cracked and degerminated, the germ separated and dealt with separately, while the endosperm and cortical portions are ground by mill-stones, and screened through "Silk" sieves. By this means the corn bran is separated from the crude starch liquors, which latter are rich in "gluten."

At an appropriate gravity (about 5° Beor sp. gr. 1·04) these crude liquors are run over the "tables," "gutters" or "runs"; which are channels about 120 feet long, 20 to 24 inches wide and 8 inches deep, made of concrete or asphalt, supported on an adjustable bed, and accurately kept level. By this means the glutinous and cellular particles are "tailed off," and the starch is deposited on the runs, from which it is shovelled off into "breakers." This process of "running" is repeated after an "alkali" treatment before it is manufactured into starch for culinary or textile purposes. A portion of the once run starch is "got up" in water, and appropriately hydrolyzed by acid for the manufacture of "glucose" in the glucose house-white. The tailings from the

silk sieves, i.e., bran, along with the glutinous tailings from the "runs," are dried in the Drying House, and sold separately, or admixed for cattle food.

The re-run starch is next put into "breakers"—tubs with strong stirring arms—, and "got up" with a minimum of water, then pumped to the "boxing houses." These are a series of perforated boxes about 6 feet long by 8 inches width and depth, standing over a trough for the collection of the drippings from them. The boxes are lined with cloths of unbleached cotton, and into these the starch is pumped, and allowed to drain "hard," after the manner in which curd is dried in the making of cream cheese. When hard the starch is broken into "lumps" and placed on trays to dry in steam-heated kilns. *Up to this point the starch being "wet starch," no dust arises from it.*

From this point, however, when the starch is dry, more or less dust rises during its handling for packing, according as it is packed in "lump," "crystal," or "pulverized" form—in which last case it is ground in appropriate mills, and screened through bolting cloth. Such is the starch that is packed by the "Packing Room Girls," of whom more later.

As already noted, the resultant product consists of little beyond starch granules; proteins and cellular matters are present in but minute amounts. Upon becoming familiar with the environment of the workers in the packing and grinding departments during their working hours, it was borne in upon me that if anywhere, here must exist the circumstances which are sure to cause deleterious effects upon the health of the employees. Not only should the particles of starch on reaching the lungs set up mechanical irritation, but, despite the treatment of maize by "sulphur water" in the steeps, the starch prepared therefrom is far from being sterile. This allows of the probability of nocardiases in addition. The remarkable fact is that despite the abundant starchy dust pervading the rooms set apart for these departments my observations soon revealed to me a singular absence of respiratory disturbances among those working in these rooms. Further I found that during the fifty years during which these particular Starch Works have been in operation there had been the same absence of pulmonary disorders among the workers.

A study of the general appearance and demeanour of the starch workers while at their duties, and during their hours of leisure, forced me to the unwilling conclusion that they enjoyed unusually good health, and were perfectly contented with their environment, during the discharge of their duties. From enquiries made of the physicians who have practised here for periods of five and eight years respectively, my

conclusions appear to be in accordance with their experience in practice.

For lack of time due to other duties, I have been unable to make any systematic physical examination of the employees of the starch house, but of sixty-five of them whom I have questioned closely as to their health, not one was aware of any ill effects, or admitted to any pulmonary or respiratory trouble attributable to their occupation, but they all—more or less—admitted to constipation, which might, in part at least, with justice be referable to the sedentary, or may I say “static” conditions under which their work is performed. I shall refer again to this point. This fact is of paramount importance, for it is well known that common labour will “kick” for no good reason, and to all outward appearances, the starch befogged atmosphere which they respire, would seem to give them good cause for complaint. It is difficult to convey the extent to which the atmosphere of the (girls) packing room is pervaded by starch. I can only state that the average amount of starch dust which settles from the air at about twelve inches below the average nose line (the girls stand at their work) is 1·875 grains per square metre in twenty-four hours. Again, in the small filling room, which is really the dust supply to the packing room, the deposition of starch at the nose line reaches 4·120 grains per square metre in twenty-four hours. I may add that fully 95 % of this starch dust settles within the seven or eight hours of their occupation, and that it is heaviest in amount nearest to the filling room (near to which the guinea-pigs about to be mentioned are encaged), gradually abating as this point is receded from. In this filling room the one pound cartons of pulverized starch have been filled by the same man continuously for upwards of eight years. He makes no complaint regarding his health, but states that he was conscious of irritation from starch dust inhalation only during the first “two or three days, while new at the job.” This is in accordance with an observation made on a guinea-pig. One was killed after living seven days in the packing room. Its lungs showed many recent (bright red) small broncho-pneumonic spots, sharply defined on the surfaces of both lungs, and which extended into the lung tissue. On section the lungs showed some bronchitis and a mild type of pneumonia, with some infiltration and the presence of large free cells in the alveoli of the type of the Staubzellen, or those of catarrhal inflammation. No starch granules were observed, even after staining with iodine, but it is fair to assume without a doubt, the broncho-pneumonic patches referred to above were caused by the irritation produced by inhaled starch granules,

even though so far, I have not been able to demonstrate them in the lung tissue. Three more guinea-pigs, exposed to the starch dust for one, two and three days respectively, and then killed, all showed a bronchopneumonic condition in proportion to the periods of their exposure to the dust inhalation. Another animal was killed at the end of fourteen days within two hours of starch dust inhalations. The necropsy showed nothing, and on section, the lung presented an extremely modified picture of the foregoing observation. Five other test animals were killed at various intervals, extending to ten months of starch dust inhalations, and their lungs far from disclosing any lesion have been strictly normal in gross appearance and under the microscope. In all cases there was no persisting catarrhal inflammation of the upper respiratory tract, though in two cases a slight catarrhal condition was observed, and I may add that the very complicated nasal structure of these animals is not calculated to permit of the easy passage of starch particles into the ultimate elements of their respiratory tract.

To further satisfy myself as to the apparent good health, certainly amounting to an immunity from pulmonary troubles, enjoyed by the workers in the starch house, through the courtesy of the Chairman and Secretary of the Edwardsburg Relief Society, I was given an opportunity of looking into the distribution and disbursements of funds for the relief of the sick since 1906.

Following the enquiry yet one step farther, I tabulated the age, height and weight of workers in various departments, appending the number of years each has been working at his job. The object of this was to obtain some direct subjective evidence of any influence the dust inhalations might have upon the physical development of those exposed to it; though they may be personally unaware of it; as compared with workers in the same factory and locality, but pursuing occupations which excluded the element of starch dust inhalation. The average from each department is appended, as also are the percentage of attendances calculated from the possible working days.

In making the comparisons of "physique" as expressed in the appended tables, that accorded to the *wet starch workers* will be seen to be considerably higher than that of the workers in other departments. This is explained by the fact that this class of work, consisting as it does of shovelling wet starch from the "runs," and of working on the boxing houses, trucking and moving starch etc., is of a more arduous nature, and demands considerable strength in comparison with the output of energy to be expended by the workers in the other departments.

It is by no means a sedentary or "static" variety of work, and for that reason should be interpreted with reserve. Under the head of "Packing Room" all of the employees are girls, and due regard should be given this in the physique comparison. This then leaves only two "departments," viz. the Drying House and the Glucose House, to be compared with the dry starch workers, and shows no disadvantage to the last named, as the following table indicates. All of the starch handled in the Glucose House being *wet*, there is no starch dust therein, but in the Drying House it may be mentioned there is quite an appreciable dust from the dried bran, and especially from the dried gluten meal, which contains about 42 per cent. of starch, and 38 per cent. of maize protein. I have made no critical study of the conditions and of the workers therein, but they are apparently in good health, as is shown by the appended table.

*Averages.*

Department	Number employed	Age	Height	Weight	Years at occupation	Attendance percentage on possible working-days	Physique expressed in lbs. per year	Physique expressed in lbs. per foot	Physique percentages taking wet starch workers as 100%
Packing Room (girls)	14	17.93	5'4 $\frac{1}{2}$	119.86	2.16	97.6	6.68	1.85	79.5
Dry Starch	24	30.62	5'7	145.5	10.96	98.06	4.752	2.171	93.4
Wet Starch	27	30.96	5'8 $\frac{1}{4}$	155.8	8.15	—	5.100	2.328	100
Drying house	16	42.75	5'7 $\frac{3}{4}$	145.7	7.31	96.90	3.410	2.165	93.05
Glucose house	23	30.95	5'7 $\frac{3}{4}$	146.56	8.65	96.73	4.740	2.1685	93.09

Withal however we have absolutely no evidence which could lead us to consider that the inhalation of starch dust under the existing conditions, even though largely admixed with (maize) "gluten" which has been dried thoroughly, at this plant at least, extending over periods of from one to forty years, and aggregating in all the departments, to as many as 829.6 years, has so far been deleterious to the health of those exposed to it.

On the other hand it is well known that a form of pulmonary disease suggesting tuberculosis and fibroid phthisis is not at all uncommon amongst millers, bakers and pastry-cooks. Among those who have reported cases of this kind are<sup>1</sup> Gerhardt. He called attention to the presence of starch in the expectoration of bakers, and to chronic disease of the lung, simulating tuberculosis, which had been excited by the inhalation of flour dust.

<sup>1</sup> Gerhardt (1896). *Centralblatt für innere Medizin*, No. 20.

Again, von Jaksch<sup>1</sup> has reported the case of a flour-mill hand in whose expectoration he found abundant starch granules which continued to be discharged after he had for two days placed the patient upon an absolutely starch free diet. The train of symptoms noted by him were similar to those depicted by Gerhardt, but with the superposition of a chronic nephritis.

It would seem from these observations that there are conditions in which starch taken into the lungs and upper respiratory tract is not immediately dissolved. Possibly this may be due to chronic inflammatory conditions set up by the irritation induced by the glutinous and cellular residues in wheat or such-like flour, which may enshroud the granules of starch, and so protect them for a longer or shorter time by the formation of an envelope relatively resistant to the action of the body juices.

I have examined 58 samples of sputum (collected after "coughing up," i.e., saliva and bronchial secretions) obtained from workers in the starch factory at different periods in relation to their exposure to starch dust inhalations:

(i) 18 samples taken as the workers were leaving the factory after a full day's exposure to the dust: in every case there was abundant evidence of starch dust.

(ii) 5 samples taken after 20 hours absence from the works: in every case maize starch granules were found embedded in theropy post-pharyngeal and laryngeal mucous secretions. The granules were mostly the smallest in size.

(iii) 31 samples taken after about 36 hours absence from the works, i.e., on Monday morning—they being absent since Saturday afternoon. In 24 cases maize starch granules were found, but were extremely few in number, in 4 cases their presence was doubtful, while in 3, they were entirely absent.

In this series the samples were taken as they entered the factory gates before going to their duties. They were all dressed in their work-a-day clothes, much starch bedusted, and it is probable that in putting them on, they must have "raised a dust," and so most probably inhaled some of the starchy particles. This is substantiated by the fact that many of the larger sized granules were in evidence, in contradistinction with (ii) the "20 hour absence" tests; for these later samples were collected from individuals dressed in their Sunday best, and on their way from church, and showed the absence of the larger granules.

<sup>1</sup> von Jaksch (1906). *Verhandl. des XXIII. Kongresses für innere Medizin, Munich.*

It must not be lost sight of however that starch cells become wafted in the general atmosphere of the village, away from the immediate vicinity of the works, and that starch bedusted workers, on their way to and from work, will also disseminate the starch granules broadcast through the village.

In regard to the four doubtful cases I have no special remarks to offer, but in the three cases in which no starch cells could be found, I afterwards learnt that two of the individuals were in the habit of changing their starch-dusted working clothes before leaving the factory, and this was doubtless contributory to the absence of starch grains in their sputum. Two more cases, absent from the factory for three days, showed very slight numbers of starch granules, but they were garbed in dusty clothes, and the granules seen were both large and small.

The last case, an individual one, and one of the men employed in grinding the starch, was away from the village for four days. The sample of sputum was taken as soon as he disembarked from the train; no starch was found therein. I may mention that in nearly all of the samples taken from the workers as they entered the factory gates on Monday morning, wheat starch—from the bread of their morning repast—was encountered, and where “starch” is unqualified above, reference is made to maize starch.

The general indication obtained throughout these microscopic examinations was that the smallest sized starch granules persisted longest in the sputum; they all stained intensely blue-black. In some cases, where the larger granules were seen, a few appeared more or less eroded, and did not stain so deeply with iodine. The general indication is that there appears to be some difference in the molecular constitution (if not composition) of the smallest and largest starch granules.

It might be mentioned that this investigation was done in the works' laboratory. By ordinary methods of washing and wiping of slides, it was found impossible to get them free from starch. Even the iodine solution in a ground glass stoppered bottle (in which it had been standing several weeks) was not starch free. It was found necessary to boil the iodine solution in order to destroy the contained starch cells, and also to keep all slides immersed in boiling water up to the time of using.

Several “blank” observations were made during the examinations reported above, so as to satisfy myself that all samples were examined under “starch free” conditions.

I must now refer to three dry starch workers—men—who do not

enjoy good health. They were ailing before taking up their present occupation. They are not aware of having become more ill since, but they are sure that their environment is not conducive to their well-being.

*Case 1.* Age 52. Had been a labourer on railway construction until three years ago, and left that occupation on account of failing health, and has worked ever since then in the starch house. Complains of pains in small of back and in groin, at times becoming very acute, and there is occasional distress on micturition. I would surmise, from what he tells me, that he suffers from "gravel."

*Case 2.* Age 32. Has never been robust, and for last eight years has been working in the starch house. Suffers from a chronic catarrhal condition of the throat. He is of a nervous temperament, and sleeps badly, awakening with a smothered feeling. Has never been troubled with a cough or other respiratory discomfort, except that above alluded to. Since he has taken more out-door exercise, and has been more careful with his diet (suffers from starch indigestion) he sleeps better, and the vicious train of symptoms is disappearing.

*Case 3.* Age 35. Has worked in the starch house for years although a victim to asthma. Drinks to excess—is in a very depressed state of vitality.

I have had no time to examine more fully into these cases, but I shall do so in the near future, when an opportunity offers.

To sum up briefly, from the foregoing it is evident that while protracted inhalations of *flour dust* induce morbid changes in the respiratory system, those of starch granules (*maize*) practically in a pure state appear to exert no such adverse influence *per se*. However, in cases where an illness is not completely recovered from, a depression of vitality is maintained, and other intercurrent diseases may supervene as a result. The reason for this is strongly indicative that flour dust, on account of its glutinous content, and the *nature* of the gluten, forms an intractable dough with the body juices, and is converted into a mass which, not being amenable to lysis, remains behind, and becoming a "foreign body," sets up irritation of the parts. On the other hand, *starch granules per se*, as in relatively pure maize starch granules, or even admixed with a very large preponderance of maize "gluten," not tending to form a dough, are easily of access to, and are rapidly removed by, some agency, and are not given the opportunity to become a foreign body, and to set up irritation.

Though the empirical chemical composition of all *starches* is identical, a few of the striking differences in chemical and physical characteristics of these two classes may be gleaned from the following:

Flour dust	Maize starch dust	Gluten meal dust
1. A high protein and cellular tissue content: the protein forming a sticky, doughy mass, with moisture.	A low protein and cellular tissue content.	A high protein, starch and cellular tissue content: the protein, however, not tending to form a dough with moisture, and the dried protein being hard and brittle, and resembling to some extent hard-wood sawdust.
2. Circular and oval granules, varying in diameter from .005 to .041 mm.	Faceted polygonal granules roughly hexagonal, varying in diameter from .007 to .023 mm.	
3. In boiling water forms a milky and relatively mobile paste, thickening up rapidly on cooling, with formation of flocculi.	In boiling water forms a relatively translucent and cloggy paste, thickening up more slowly and with less marked flocculation.	

Without going farther into these chemico-physical differences, let us turn our attention to the mechanism by which the starch granules are removed, after gaining entrance to the lower respiratory passages.

It is well known that the blood contains an amylolytic enzyme which, according to Halliburton<sup>1</sup>, is said to convert starch into iso-maltose,—and incidentally I may mention the entity of iso-maltose is, to my mind, somewhat undefined, and savours of a malto-dextrin, or of “gallisin”? This diastatic enzyme in the blood has been shown to increase during constipation and to decrease during diarrhoea, and is held to have its origin from the pancreas. I have mentioned previously the fact that the dry starch workers complain of constipation, and I am told by the two practitioners here, and by one in Prescott, that they have had cases of dyspepsia from amongst them which have shown marked inability to digest starchy foods. Is this constipation due to a call for an amylase in the blood to digest the inhaled starch? By so deflecting that pancreatic enzyme from its normal channel, is “starch indigestion” caused, and constipation engendered by an induced quiescence of the bowel? The inhaled starch, if digested by an amylase in the blood,—is it utilized by the organism, or is it voided through the urine before its oxidation is complete? I have examined upwards of fifteen samples of urine from different individuals, and have invariably found the specific gravity, colour, odour and reaction to litmus and lacmoid papers normal. No trace of sugar, acetone, or diacetic acid has been found in any one of them, but they all without exception

<sup>1</sup> Halliburton, *Essentials of Chemical Physiology*, 4th Edition, p. 141.

show up indican in smaller or larger amounts, indicating thereby lack of normal intestinal activity and tone.

Anent the inhalation of starch dust, Parkes and Kenwood, in their *Practical Hygiene*, 3rd edition, p. 185, state: "Millers and bakers are liable to inhale flour dust, but as this substance is probably arrested in the mouth and nose, and does not reach the lungs, it can hardly be regarded as productive of lung disease." Because of the very excessive quantities of dust to which such workers (starch packers included) are exposed, is the flour or starch dust not liable, so to say, to form an incrustation over the tubulure of the upper respiratory system, which will present a fairly "*dry*" surface, incapable of collecting after a while, any more flour or starch dust, and so eventually allowing these particles to reach the alveoli? Though starch granules have not been demonstrated in the guinea-pigs' lungs in the four tests made, a bronchopneumonia has supervened. Some of the hands tell me that the starch "*dries up*" *their saliva and bronchial secretions*, and that they cough up lumps of slime with starch four and five days after being away from the factory. A few have also told me that for relatively long periods after they have been away from the works, starch seems to "*come* from the pores of their skin." These matters I hope to be able to investigate during our four week midsummer shut down.

Moscati of Naples<sup>1</sup> has observed the destruction of starch pastes injected intravenously and subcutaneously into the organism, and suggests the probability of glycogen being formed from the starch so injected. This idea of anabolism, or rather for the want of better terms, I should say "*anabolic Katamerism*," or depolimerization of starch (pastes) into glycogen I cannot support. It appears to be heterodox to expect that the organism should *reorganise and store for future use a body unnaturally and a-topically "invading" it, and hence a foreign body, when its every effort should be, in the light of our present thought, to break it down, to simplify and to annihilate it and throw it out.* He does not seem to take into account the degradation of the starch pastes through the "*amylo-*" and "*erythro-*"-dextrins into its final reducing sugar, and the fact that at a period in a diastatic starch conversion, the classical mahogany-purple-brown iodine reaction of glycogen is arrived at, (at about  $[\alpha]$ ) and may be misleading under certain conditions, and especially in the presence of proteids. However, he conclusively shows that the organism is endowed, not only in its blood, but in its various

<sup>1</sup> *Zeitschrift f. Physiol. Chemie*, vol. L. p. 73.

organs and tissues, (possibly through the blood serum therein) with an abundant normal amylolytic power.

He does not suggest the probability of the exaltation of the amylolytic power of the organism to combat (as an anti-body) the invasion of starch. To establish or disprove this has been the cardinal idea of the investigations made through the courtesy of Professor Adami in his laboratories by Dr A. C. Rankin, and by myself here in the laboratories of the Edwardsburg Starch Company. The results of these experiments were conflicting, though interesting, and our data do not allow any definite statement on this point to be made in this paper, nor have we enquired into the nature of the final sugar produced. Parallel series of blood examinations for amylolytic power were conducted on men, rabbits and guinea-pigs, which on the one hand had been subjected to the inhalation of starch granules, or injections of starch pastes, and upon the other, those which were not so exposed. But in our controls, such large variations were noted among different individuals, and even in the same individual at different times, as to render the information obtained useless for the establishing of a "normal." Similar results were noted in the experiments, though upon two occasions it seemed as though a distinctly great exaltation in amylolytic power was observed as being due to the exhibition of starch. An explanation of these irregular results may reasonably be sought in the relative importance of the digestive ferments to animals of differing proclivities in regard to their diet. For instance, if we grant that the enzymic activity of the blood and other body fluids is the expression of the degree of diffusion or seepage into it from the glands properly detailed to secrete these ferments, then in the blood of herbivora and graminivora (guinea-pigs, rabbits etc.) we should find a high normal amylolytic, and correspondingly low proteolytic power. In omnivora (man etc.) we should expect to find a relatively smaller amylolytic, but higher proteolytic power of the blood, while in carnivora we should reach the other extreme, and therefore find that their blood serum was rich in proteolytic ferments, but low in amylolytic. These are matters which require further investigation.

In a letter (Feb. 12th, 1908) I wrote to Prof. Adami telling him of my views regarding the probability of an exaltation in the amylolytic powers in the blood of those who are exposed to starch dust inhalations, I mentioned the fact—which was corroborated by our local practitioners—that while there were several cases of malignant new growths (both carcinoma and sarcoma) in our village, it was remarkable that no case of malignant disease had ever been observed in persons employed amongst

starch dust, though cases had been known to exist amongst the out-of-door group. Upon purely casuistic grounds I discussed the matter from the standpoint of Beard's trypsin treatment for cancer.

About eight months later, while working independently, Dr A. A. Bruère of the Clinical laboratory of the Royal Victoria Hospital, I am told by Dr Adami, found an exceptionally well marked amyloytic activity of blood drawn from a patient then diagnosed to be suffering from a mediastinal lympho-sarcoma. The diagnosis was confirmed at autopsy.

I am grateful to Professor Adami for the interest he has taken in my ideas, for his kind direction, and for the many facilities accorded me; to Dr A. C. Rankin I am also greatly indebted, for much unselfish work done on my behalf. Lastly, I must thank Mr G. F. Benson, President of the Edwardsburg Starch Company, for the unhampered facilities allowed me in obtaining data, and for the use of the excellent laboratories of the Company at Cardinal.

## OBSERVATIONS ON THE INFLUENCE OF HEATING UPON THE NUTRIENT VALUE OF MILK AS AN EXCLUSIVE DIET FOR YOUNG ANIMALS.

By JANET E. LANE-CLAYPON, M.B., D.Sc.,

*Jenner Memorial Scholar, Lister Institute of Preventive Medicine.*

(1 Figure.)

DURING the year 1904, a year which included four months of hot summer weather, the mortality among infants under one year old in England and Wales was 145·3 per 1000 births. Of this mortality 21·8% died of diarrhoeal diseases and 31·5% from wasting diseases.

Experience shows that these troubles affect hand-fed to a much greater extent than breastfed infants.

Thus, in Paris, in 1898, from the 14th to the 27th of August 550 children died of diarrhoea. Of these 57 are recorded as being breast fed and 493 as artificially fed (Budin, 1907).

In Rouen during 1900 to 1905 out of 550 babies attending a large dispensary 57% were breast fed (1), 7·4% were on breast and artificial feeding (2), 35·6% on artificial feeding only (3). Of these 7·6% of (1) died, 26% of (2) and 35·6% of (3) (Budin, 1907).

Similar statistics have been published for Lille, Elboeuf and other places.

There are no available statistics of any large number of babies in England, but in Finsbury out of 212 infant deaths 21 were due to diarrhoea; of these none were breast fed, 4 were on breast and other foods and 17 were artificially fed (Finsbury Report on Public Health for 1907).

A large proportion of this infantile mortality must be attributed to a harmful or faulty nutriment, and as the diet of children of less than one year old is practically confined to milk, the remedy consists in securing for the infants innocuous milk of adequate nutritive value.

Much of this continual waste of infant life, is no doubt due to bacterial contamination of the milk, a danger which can be nullified by the heating of the milk shortly before its use. Provided that the temperature is sufficiently high and the length of time it is maintained adequate, the destruction of micro-organisms can be ensured, but, to effectively reduce the mortality from infected milk the remedy must be one which can be carried out in the poorest tenement lodging, that is, one which demands a minimum of skill and necessitates no special apparatus.

The raising of milk to the boiling point and allowing it to cool with some sort of covering fulfils these conditions. Why is this simple precautionary measure not put into practice? The reason is, I believe, due to a widespread belief in this country that the boiling of milk deprives it of some properties essential to the infant's well-being. Many medical men are convinced that rickets, scurvy and other troubles follow the use of boiled milk. Infantile scurvy seems, however, to be an uncommon disease and not unusually has been caused by feeding on some patent farinaceous baby-food.

In Paris, where several thousand babies are fed every year on milk that has been boiled for 20 minutes, rickets is rare and scurvy almost unknown; thus Budin (1907, p. 198) has not seen a single case of scurvy among children fed on such milk, and his experience is borne out by Bresset (1888—1905) who has one of the largest milk dispensaries in Paris.

During recent years a mass of valuable evidence has been accumulated in France showing that a diet of milk which has been boiled for 45 minutes gives excellent results.

Budin (1907, p. 173) gives curves showing that the weight of children fed on sterilised milk is almost identical with that of a normal breast fed baby. This does not show that sterilised milk is a desirable substitute for the breast, since an artificially fed child is always in danger of digestive troubles, but it shows that, granted the necessity for artificial feeding, boiled milk is a suitable substitute. A few dispensaries in France use pasteurised milk for the artificially fed infants, but although I have been unable to obtain any published statistics, I understand that the results have not been superior to those obtained with sterilised milk. Nor are there any weight curves available for comparison of babies fed on raw milk, although a few experiments were made by Bresset who did not find it in any way superior to sterilised milk for normal children.

The question of the modification of the nutrient properties of milk by boiling has often been attacked from the experimental side, but owing to the care required in bringing up young animals upon a foreign milk, boiled or unboiled, and the small number of animals used for the experiments, the results are not so satisfactory as could have been wished.

Bolle (1903) states that guinea-pigs fed on boiled milk got Barlow's disease, whereas those fed on raw milk did well. Guinea-pigs are however not suitable for these experiments, and the results have not been confirmed by Bartenstein (1905). This last observer also fed dogs on boiled and preserved milk (two on each). He does not consider that there was any evidence of ill-health, but there appear to have been complications.

Keller (1904) fed mice on raw, boiled, and sterilised milk, and found that all did equally well; young dogs fed both on fresh or sterilised milk also did equally well, no difference being detected after three months.

Price (1904) found that calves fed on raw milk gained weight, much better than on sterilised milk. Whilst fed upon the latter they had diarrhoea. The experiment was only carried on over 8 days for each kind of milk, and moreover, some calves which did badly on raw milk, subsequently improved on pasteurised milk. Price considers the digestibility to be slightly impaired by boiling.

Peiper and Eichoff (1904) found that dogs became anaemic and the bone rarified after prolonged feeding on slightly sterilised milk.

The experiments of Brüning (1906) are especially noteworthy; he finds that when the milk of a foreign species is used, better results are obtained with boiled than with raw milk, and this as a result of prolonged experiments on pigs (omnivora), dogs (carnivora), and rabbits (10 days experiment), guinea-pigs (14 days experiment) and goats (herbivora).

The experimental evidence, therefore, although not absolutely concordant, certainly seems to show that when the milk of another species is used, there is no marked nutritional difference between raw, boiled, or even sterilised milk, a result which the observations about to be described entirely confirm.

#### *Experiments.*

Rats were selected for our experiments. The animals were as nearly as possible a fortnight old at the commencement of the

experiment. They were kept in batches of a dozen in airy cages sufficiently large for them to obtain plenty of exercise in climbing about the wires. They were fed twice a day throughout the experiment—morning and evening—on bread and milk, both bread and milk being weighed daily, and adjusted as nearly as possible, so that, although they had plenty to eat, there should be no stale food left in the cages.

The rats in each case were weighed daily all together; in the following figure the results were obtained by dividing the weight by the number of rats.

*Series A* received bread and milk from the Walker Gordon Laboratories delivered fresh and kept at 0° C. in the cold room until just before use.

*Series B* received bread and the same milk as Series A, but raised to 96° C. just before using.

*Series C* received bread and sterilised milk. The sterilised milk used was made from the full-cream dried milk of the West Central

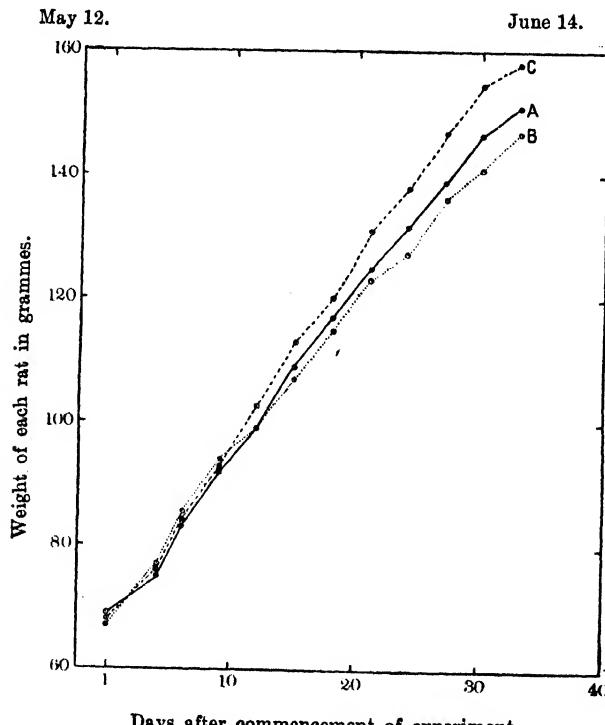


Fig. 1. Showing growth of rats in grammes.

Surrey Dairy, in a 14% solution, this being almost precisely the average composition of cow's milk.

The details of the results are given in the Table.

*Table showing weight of rats used in Series A, B, C.*

Date	Series A (Raw milk)			Series B (Boiled milk)			Series C (Sterilised milk)			Food in % of body weight	
	Total wt. in grams	No. of rats	Average wt. per rat	Total wt. in grams	No. of rats	Average wt. per rat	Total wt. in grams	No. of rats	Average wt. per rat	Bread	Milk
May 12	837	12	69·7	800	12	66·6	815	12	67·9	20 %	30 %
,, 15	900	12	75·0	919	12	76·5	912	12	76·0	—	—
,, 18	1002	12	83·5	1027	12	85·5	930	11	84·5	—	—
,, 21	1107	12	92·2	1120	12	93·3	1020	11	92·7	—	—
,, 24	1187	12	98·9	1185	12	98·7	1132	11	102·9	16	24
,, 27	1310	12	109·1	1285	12	107·0	1242	11	112·9	—	—
,, 30	1403	12	116·9	1382	12	115·1	1322	11	120·1	—	—
June 2	1490	12	124·1	1470	12	122·5	1438	11	130·7	13	20
,, 5	1582	12	131·8	1552	12	129·3	1820	11	138·1	—	—
,, 8	1670	12	139·1	1632	12	136·0	1625	11	147·7	11	16
,, 11	1755	12	146·2	1688	12	140·6	1710	11	154·5	—	—
,, 14	1820	12	151·6	1770	12	147·5	1740	11	158·1	10	15
Increase % = 217·5			Increase % = 221·5			Increase % = 232·8					

Of the three dozen rats, one died in Series A a few days after the beginning of the experiment. Of the others it would be difficult to say which batch seemed the healthiest: they all did splendidly: no evidence was obtained of any digestive disturbance; the coats of all were in perfect condition, and the rats exceptionally fine. That they were neither anaemic nor rickety was sufficiently evidenced by their great agility in climbing about the cages, nor was any evidence of such obtained by examination.

The experiments were brought to an end by the detection of an early pregnancy in one of the rats of Series C, evidently about the twelfth day; 12 days were therefore knocked off the experiment, so that no complications appear on the curve here given. About a week after the discovery of the first pregnancy, nearly all the females in all three series were found to be pregnant: they went to term and had large families of healthy rats.

In Figure 1 it will be seen that the rats of Series C (sterilised milk) gained slightly more weight than the other series. The

curve of Series *A* rises higher than *B*, but the initial weight was greater; the actual gain per cent. of *B* was greater than that of *A*, as shown in the table. In another series of experiments also on rats, with raw and boiled milk, the rats fed on raw milk did slightly better as regards weight than the series on boiled milk, but two died of each lot and the rats fed on boiled milk had what appeared to be a nasal catarrh for some weeks during the experiments, which probably contributed to the slight difference in weight: both series were in splendid condition, and all the females had large and healthy families at the age of about eleven weeks.

There would, therefore, appear to be no diminution in nutritive properties for rats, by boiling or even evaporating and drying the milk at 120° C.

#### REFERENCES.

- BARTENSTEIN (1905). *Jahrb. f. Kinderh.* p. 61.  
BOLLE (1903). *Zeitschr. f. diät. u. physik. Therapie*, Vol. 6, p. 354.  
BRESSSET (1888-1905). *Le Dispensaire de la Caisse des Écoles du VII. Arrondissement.* Statistique de 1888-1905, pp. 25 and 26.  
BRÜNING (1906). *Zeitschr. f. Tiermed.* No. 110, pp. 198 and 277.  
BUDIN (1907). *L'Hygiène de l'Alimentation*, Paris, pp. 157-158 and pp. 173 et seq.  
KELLER (1904). *Zeitschr. f. diät. u. physik. Therapie*, Vol. 7, p. 90.  
MORO (1907). *Münchener med. Wochenschr.* No. 45, p. 2223.  
PEIPER AND EICHOFF (1904). *Korresp. d. Ver. d. Ärzte im. Reg.-Bez. Stralsund*, 1904.  
PRICE (1904). *New York Med. Journ.* Vol. 79, p. 405.

MULTIPLICATION OF BACTERIA AND THE INFLUENCE  
OF TEMPERATURE AND SOME OTHER CONDITIONS  
THEREON.

By JANET E. LANE-CLAYPON, M.B., D.Sc.

*Jenner Memorial Scholar, Lister Institute of Preventive Medicine.*

1 Figure.

THE methods which have been employed for estimating the rate of growth of bacteria are numerous.

Nägeli (1887) endeavoured to estimate the rate of growth by studying the amount of acid produced by an acid-producing organism.

Buchner, Longard and Riedlin (1887) studied the rate of growth of the cholera bacillus, using the plating-out method of Koch. They plated-out the culture at the beginning and at the end of a period of from 2—5 hours. The generation time was calculated from the formulae

$$2^n = \frac{b}{a} \text{ where } a = \text{No. of bacteria at beginning,}$$

$b = \text{No. of bacteria at end}$

and

$n = \text{No. of generations,}$

and  $G = \frac{T}{n}$  where  $G = \text{generation time and } T = \text{time of experiment.}$

The results obtained for  $G$  varied from 19 to 40 minutes at 37° C. This appears to be a large difference, but, as pointed out by Hehewerth (1901), Buchner did not allow for the initial "lag" which most observers find when a fresh culture is made.

Rahn (1900), working chiefly with *B. fluorescens*, has studied the question of this early lag; he found that the minimum generation time at 24° C. occurred 6—21 hours after inoculation. After this the growth was very slow up to about 48 hours.

Klein (1900) counted the number of stained bacteria in a given field, knowing the area of the field in its relation to the area covered by a drop spread out on a film.

Hehewerth (1901) published a number of experiments on the growth of *B. coli* and *B. typhosus*. He worked with large numbers of bacteria, the first estimation generally giving a count of several millions.

He found that there is an initial period after inoculation during which growth is almost absent : the length of time of this "lag" varies with the age of the culture used for inoculation and with the species of the bacillus. With a 19 hours old culture of *B. coli*, the lag at 37° C. lasted rather over one hour : with *B. typhosus* it lasted over two hours.

A culture having once started growing, the numbers run up quickly and then remain fairly constant for some time, subsequently slowly decreasing.

The generation time of *B. coli* at 37° C. in broth he found to be 21—31 minutes, the average being 23 min. 24 sec. and in peptone water to be 28—32 minutes, or sometimes rather more, depending upon the age of the culture. The generation time for *B. typhosus* averaged 33 min. 24 sec. in broth, and 45 min. 37 sec. in peptone water.

Boland (1902) tried to estimate rate of growth by using a standard turbidity.

Müller (1903) used cultures of bacteria isolated by him from frozen material : with slight variations the generation times were the same for the different bacteria at the same temperature, viz., about 50—60 minutes at 30° C. during the first 12 hours, with a lag of nearly two hours ; and about 54—70 minutes at 25° C. with a lag of about three hours, or rather more.

He usually started the experiments with a culture containing about 1000—2000 bacteria per c.c.

Barber (1908) worked chiefly with *B. coli*. By means of a fine capillary pipette he removed a single bacterium and determined the actual rate of division. He found no preliminary lag if the bacteria were inoculated into a medium to which they were accustomed. He found that the generation time gradually decreased up to about 40° C., after which it increased.

For *B. coli* he obtained the following times with individual variations of several minutes :

20° C. ....	60 mins.	40° C. ....	17 mins. or rather more.
25° C. ....	41 mins.	42° C. ....	19—20 mins.
30° C. ....	29·7 mins.	45° C. ....	30—34 mins.
37° C. ....	17—21 mins.	50° C. ....	no growth.

His figure for 37° C. is lower than that of other observers.

The rate of multiplication of bacteria increases from 2—3 times between the temperatures of 20° C. and 30° C. (cp. Hchewerth and Barber).

The optimum temperature differs for different species, and the temperature at which they are kept as stock cultures in the laboratory has been found to have some influence in determining the subsequent rate of growth at any particular temperature.

### *Methods.*

Throughout these experiments the same method has been used, the details being reproduced each time as far as possible.

The cultures used had all been kept at room temperature for over one year. The species of bacteria used were *B. coli*, *B. typhosus* and *B. enteritidis* Gaertner. A fresh agar culture was made and kept

at room temperature for 24 hours and a sub-culture was made from this into about 5 c.c. of broth medium.

The broth culture was then allowed to grow for about 20 hours also at room temperature. With the organisms employed, it was found by experiment that a millionfold dilution of this culture afforded a suitable number of organisms to use for the beginning of an experiment, viz., 200—500 bacteria per c.c.<sup>1</sup>

The culture was kept in the dark at the desired temperature. The temperature varied within half a degree centigrade of that recorded.

At intervals after incubation a definite number of drops (1 drop = .02 c.c.) were removed by means of a standard capillary pipette and plated.

The actual number of drops required to produce a reliable plate at different periods of incubation had to be discovered by experiment. Two or three plates were made for each estimation.

In the later stages of the experiments the culture required dilution before plating; this was carried out as rapidly as possible by dropping one drop of the culture into the required amount of sterilised water, shaking well, and then with another similar pipette the desired number of drops of the dilution were plated as usual. The whole time required was about two minutes, and control experiments showed that there was no deleterious effect from the distilled water during this short time.

### *Results of Experiments.*

The observations show that there are four phases in the bacterial life of a culture:—(1) an initial period of slow or of no growth; (2) a period of regular growth, the rapidity varying slightly at the same temperature, but differing widely for different temperatures; (3) a period when the numbers remain more or less stationary; (4) a period when the numbers of living bacteria are diminishing.

#### *Period I.*

All observers, except Barber (1908), record an initial lag varying in extent at different temperatures. In all my experiments I found a very definite lag, during which the number of bacteria per drop remained almost constant.

<sup>1</sup> The number of organisms mentioned throughout this paper refers to estimates formed from plating out on agar. This method does not give 100% of the organisms present but with the same sample of culture medium affords a constant error.

With *B. coli* and *B. enteritidis* Gaertner this latent period was found to extend from one to six hours as the temperature varied from 42° C. to 20° C. In the case of *B. typhosus* the lag was rather longer at each temperature.

This lag agrees almost exactly with that observed by Hehewerth (1901) who used cultures of very nearly the same age (19 hours). Rahn (1906) states that it is less if the inoculation is fairly heavy.

### *Period II.*

The lag being over the bacteria now enter upon a phase of rapid growth.

The rate of growth has been calculated by various observers on the assumption that the bacteria are all in a state of active and regular division so that the number increases logarithmically. By the method described, I have been able to ascertain that this is the case for a considerable length of time after the culture first starts growing.

The actual figures showing the increase in numbers of *B. coli*, *B. typhosus* and *B. enteritidis* Gaertner, at temperatures 20—42° C. are set out in Tables I, II and III respectively. In parallel columns are placed the average logarithmic differences per hour, the proportional increase in number per hour and the mean generation times as calculated<sup>1</sup> from the observations.

In Fig. 1 the logarithms of the numbers of *B. coli* found in unit volume at different intervals have been plotted against time. This has been done for various temperatures and the points fall upon straight lines. The slope of these lines is different at the various temperatures, the rate of growth being proportional to the tangent of the angles made by them with the abscissa.

Similar graphs are obtained if the figures for *B. typhosus* and *B. enteritidis* Gaertner be also plotted in the same way.

<sup>1</sup> During the time that growth proceeds logarithmically the number of generations in any interval of time  $t_1 - t$  is measured by the power to which 2 must be raised to produce the same increase as the proportion between the number of bacteria at time  $t_1$  and  $t$ .

$$2^n = \frac{\text{number at } t_1}{\text{number at } t};$$

taking logarithms the equation can be expressed:

$$n = \frac{\log \text{number } t_1 - \log \text{number } t}{\log 2};$$

$n$  is the number of generations in time  $t_1 - t$  and the generation time is  $\frac{t_1 - t}{n}$ .

In the experiments in the tables, the ascertained period of "lag" was allowed to elapse before the first observation was made.

For a given volume of culture fluid the time during which the bacteria continue to divide at a maximum rate depended upon the insemination and the temperature, being shorter if the inoculations were heavy and at the higher temperatures.

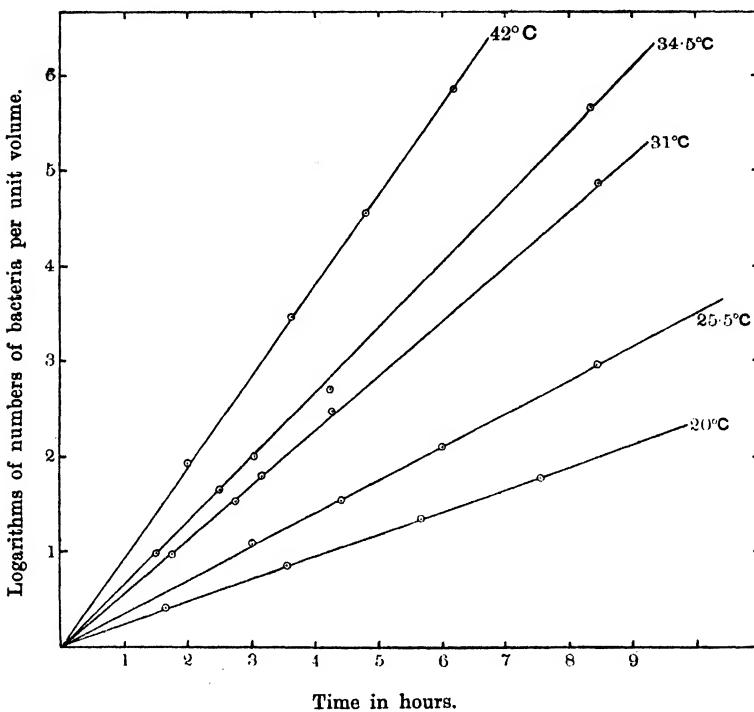


Fig. 1.

The results recorded below are those obtained with an insemination of 200—500 per c.c. Under these circumstances, and discounting the period of lag, the length of time over which *B. coli* and *B. enteritidis* Gaertner maintained the maximum rate was approximately:

7½	hours at	37° C.
8—8½	"	30° C.
12—15	"	25° C.
20—24	"	20° C.

*B. typhosus* grew more slowly and the logarithmic increase persisted longer at all temperatures.

At 20° and 25° C. the logarithmic increase was maintained almost up to the time when the medium contained a maximum number of living bacilli, but at the higher temperatures the rate fell off considerably before this point was reached.

### *Periods III and IV.*

After the culture has ceased growing logarithmically the rate of growth slackens gradually, but is still fairly active until the number of bacteria reaches several million per drop ('02 c.c.), that is several hundred million per c.c. At this stage the number of living bacteria present appears to remain fairly constant for some time (from two to five days according to the temperature) after which it begins to decrease slowly.

The results obtained with *B. coli* resembled those of *B. enteritidis* Gaertner, except that the maximum number obtained is about double.

There appears therefore to be a maximum number of bacteria which a unit volume of medium is capable of supporting: this means that for that particular organism the conditions are no longer favourable for increased growth. This may be due to the using up of some constituents of the broth or it may be due to some inhibitory substance produced by the organisms themselves in the process of metabolism. I have not carried out any investigations on these points. 400,000,<sup>11</sup> to 800,000,000 per c.c. of broth seems to be the maximum for *B. coli* and *B. enteritidis* Gaertner.

After two days at 37° C. and sometimes before, it is difficult to get an accurate estimation as there is a great tendency to agglutinate, especially with *B. coli* and *B. enteritidis* Gaertner.

### *Influence of temperature upon generation time.*

The effect of temperature was studied over the range between 20 and 50° C. Up to 42° the generation time was reduced, but above this it apparently increased, and at 50° diminution replaced increase in the number of organisms.

The experiments of Hehewerth (1901) and Barber (1908) showed that the generation time was reduced one-half to one-third by raising the temperature from 20° to 30° C. This is an effect of the same order as that of temperature upon many common chemical reactions. The logarithms of Barber's values for mean generation times between 20° and 37° C. plotted against temperature fall upon a straight line,

showing that each rise of one degree throughout this range produces the same proportionate effect.

My observations for *B. coli* and *B. typhosus* are in accord with those of Barber. The influence of temperature upon the growth of *B. enteritidis* Gaertner I find to be decidedly less. The temperature effect upon the growth of these three organisms is fairly constant between 20° and 35° C. Each rise of one degree produces the same proportionate increase in the rate of growth. The logarithms of the mean generation times (20°—35°) from Tables I, II and III plotted against temperature show a linear relationship, signifying that the effect of temperature upon the growth of these bacteria accords with Arrhenius-Van 't Hoff law within the error of the experiments.

The increase in rate of growth per 10° rise in temperature obtained by the drawn lines is 2·2 for *B. coli* and *B. typhosus* and 1·7 for *B. enteritidis* Gaertner.

Above 35° C. the effect of temperature in diminishing the mean generation time is, as found by Barber, distinctly less.

Now

#### *Summary.*

The species of organisms used were *B. coli*, *B. typhosus* and *B. enteritidis* Gaertner. With these organisms:

(1) When a fresh broth culture is made with a small inoculation there is a period during which there is no increase in the number of bacteria present.

(2) When this period is over the bacteria commence to divide regularly; this is shown by the fact that the logarithms of the numbers plotted against time are found to fall on a straight line. This regular growth persists until (or nearly until) a maximum has been reached, after which the numbers remain more or less constant and then slowly decline.

The time necessary for a complete division to take place (generation time) was determined for various temperatures between 20° C. and 42° C.

(3) The effect of temperatures between 20° and 35·3° C. upon the rate of multiplication is in accordance with the Arrhenius-Van 't Hoff law; above this temperature the effect diminishes.

I have much pleasure in thanking Dr C. J. Martin for his invaluable assistance and interest throughout this research and also Miss Müllenbach for kindly preparing the diagram.

TABLE I.

*Growth of B. coli at temperatures 20° to 42° C.*

Temp.	Time after commencement of ex- periment		No. of bacteria per drop	Logarithm of number per drop	Average Log. difference Per hour = velocity constant	Proportional in- crease in num- bers per hour	Mean generation time in hours
	hrs.	mins.					
42° C.	2	0	87	1.94	.94	8.7	.32
	3	40	2,876	3.46			
	4	50	36,675	4.56			
	6	10	739,200	5.87			
34.3° C.	1	30	9	.97	.68	4.8	.44
	2	30	43	1.63			
	3	5	105	2.02			
	4	15	499	2.70			
	8	22	476,666	5.68			
31° C.	1	45	10	1.00	.57	3.7	.53
	2	45	34	1.53			
	3	10	65	1.81			
	4	22	288	2.46			
	8	27	72,533	4.86			
25.5° C.	3	0	12	1.08	.35	2.2	.86
	4	25	33	1.52			
	6	0	128	2.11			
	8	27	893	2.95			
20° C.	12	15	243	2.38	.23	1.7	1.30
	14	10	720	2.85			
	16	15	2,166	3.33			
	18	10	5,066	3.70			
	22	30	46,666	4.67			

TABLE II.

*Growth of B. typhosus at temperatures 20°—34.3° C.*

34.3° C.	1	30	7	.84	.55	3.5	.55
	2	30	24	1.38			
	4	0	152	2.18			
	9	20	195,000	5.29			
	26	20	4,133,000	6.62			
31° C.	1	50	7	.84	.40	2.5	.75
	2	45	17	1.23			
	4	30	82	1.91			
	9	25	13,600	4.13			
	26	25	3,968,000	6.98			
25° C.	3	50	10	1.0	.23	1.7	1.30
	6	10	36	1.56			
	9	35	178	2.25			
	26	30	3,566,000	6.55			
20° C.	6	15	10	1.0	.18	1.5	1.67
	8	37	28	1.45			
	9	38	86	1.56			
	26	35	51,145	4.71			
	54	10	4,933,000	6.69			

TABLE III.

*Growth of B. enteritidis Gaertner at temperatures 20° to 42° C.*

Temp.	Time after commence- ment of ex- periment		No. of bacteria per drop	Logarithm of number per drop	Average Log. difference per hour = velocity constant	Proportional in- crease in num- bers per hour	Mean generation time in hours
	hrs.	mins.					
42° C.	2	20	83	1.92	.81	6.5	.37
	4	0	1,940	3.28			
	5	0	13,417	4.12			
	6	20	176,400	5.24			
34.3° C.	1	30	13	1.12	.59	3.9	.51
	2	30	43	1.63			
	3	0	80	1.90			
	3	30	179	2.25			
	6	15	8,960	3.95			
	12	25	3,580,000	6.55			
	25	30	5,366,000	6.72			
	49	40	10,833,000	7.03			
	121	0	6,266,000	6.79			
31° C.	1	45	13	1.11	.54	3.5	.56
	2	45	44	1.64			
	3	15	85	1.92			
	6	35	4,050	3.60			
	8	15	44,000	4.64			
	12	30	1,480,000	6.17			
	25	30	9,000,000	6.95			
	49	40	8,400,000	6.92			
	121	0	4,600,000	6.66			
26° C.	4	0	25	1.40	.42	2.6	.71
	4	45	57	1.75			
	5	30	112	2.04			
	6	15	208	2.31			
	6	45	361	2.55			
	12	0	56,933	4.75			
	25	30	9,125,000	6.96			
	49	40	9,600,000	6.98			
	121	0	10,466,000	7.01			
20° C.	6	40	28	1.44	.29	2.0	1.0
	7	45	56	1.75			
	8	35	98	1.99			
	12	45	1,546	3.18			
	26	0	7,581,400	6.87			
	49	40	10,433,000	7.01			
	121	0	10,066,000	7.00			

## BIBLIOGRAPHY.

- BARBER (1908). The rate of multiplication of *B. coli* at different temperatures. *Journ. of Infectious Dis.* v. 379.
- BOLAND (1902). Inaug. Dissert. Amsterdam.
- BUCHNER, LONGARD u. RIEGLIN (1887). Über die Vermehrungsgeschwindigkeit der Bakterien. *Centralbl. f. Bakt.* I. Abt. Vol. II. p. 1.
- HEHEWERTH (1901). Die mikroskopische Zählungsmethode der Bakterien von Alex. Klein, und einige Anwendungen derselben. *Arch. f. Hyg.* xxxix. 321.
- KLEIN (1906). Eine neue mikroskopische Zählungsmethode der Bakterien. *Centralbl. f. Bakt.* I. Abt. Vol. xxvii. p. 834.
- MORGAN (1906). Upon the Bacteriology of the Summer Diarrhoea of Infants. *Brit. Med. Journ.* I. 908.
- MÜLLER (1903). Über das Wachstum und die Lebensfähigkeit der Bakterien, sowie den Ablauf fermentativer Processe bei niederer Temperatur, unter spezieller Berücksichtigung des Fleisches als Nahrungsmittel. *Arch. f. Hygiene*, xxxvii. 127.
- NÄGELI (1877). *Das Mikroskop*, 2nd Aufl. Leipzig, p. 461.
- RAHN (1900). Über den Einfluss der Stoffwechselprodukte auf das Wachstum der Bacterien. *Centralbl. f. Bakt.* II. Abt. Vol. xvi. p. 417.

## THE PHYSIOLOGICAL EFFECT OF COBALT CARBONYL VAPOUR.

By H. W. ARMIT.

*From the Lister Institute of Preventive Medicine.*

THE discovery of cobalt tetra-carbonyl by Dr Hirtz and Mr Cowapp in Dr Ludwig Mond's laboratories offered a further opportunity of studying the toxic action of volatile compounds of the heavy metals. Through the kindness of Dr Mond, a sufficient quantity of cobalt carbonyl was placed at my disposal for physiological experimentation.

Cobalt carbonyl is a crystalline substance of red colour. It is not very volatile and the vapour has not so characteristic a smell as that of the carbonyls of nickel and iron.

The following experiments were conducted for the purpose of obtaining indications of the volatility as well as of the dissociation of cobalt carbonyl in dry air. Air, freed from carbonic acid by washing in solutions of caustic potash and dried by passing over calcium chloride and through sulphuric acid, was conducted through a tube containing crystals of carbonyl of cobalt kept at a constant temperature by means of a water jacket. The air was then passed through a glass chamber guarded by plugs of cotton wool at the outlet and inlet and thence bubbled through bromine water in a wash bottle and a Meyer's apparatus.

The cobalt found in the bromine water added to that deposited in the plugs and on the walls of the glass chamber corresponded to the amount volatilised, but it was found that, with the current of air employed, saturation was not obtained, unless the temperature of the water jacket was some degrees higher than that of the rest of the apparatus. When volatilised at 0° C. the volume percentage of the carbonyl in a current of air not exceeding 2 litres per minute was very small indeed. The maximum obtained was less than 0·001 volume

per cent. At 16° C. the greatest amount of cobalt carbonyl evaporated corresponded to about 0·01 vol. %, but of this 50 % was found as a solid cobalt compound in the wool, etc. It therefore appeared to be unlikely that a mixture of the vapour in air of sufficient concentration to produce acute poisoning in animals would be obtained by such means.

Previous experiments ("The toxicology of nickel carbonyl," *Journ. of Hygiene*, 1907, VII., 4 and 1908, VIII., 5) have shown that at least 15 mgrs. of cobalt per kilogram body weight must be absorbed to kill rabbits. A rabbit weighing two kilograms would therefore require to inhale and absorb approximately 5 c.cm. of cobalt carbonyl vapour. If exposed for two hours at 16° C. to a 0·01 vol. % mixture, 7·2 c.cm. would be contained in the air breathed, but the experiments referred to above show that not less than one half of the quantity of the carbonyl is dissociated, even in the presence of dry air. In the presence of moist air, containing carbonic acid, the amount of dissociation would be greater.

It has been shown that when an animal breathes a mixture of nickel or iron carbonyl and air only part of the vapour breathed is absorbed by the lungs. The expired air, as is the case with other vapour mixtures, contains a certain amount of the vapour. In the case of  $\text{Ni}(\text{CO})_4$ ,  $\text{Fe}(\text{CO})_6$ , CO and some other gases, the proportion of vapour absorbed has been found to be about 50 % of the total quantity. It therefore appears that even after two hours' inhalation not more than 1·8 c.cm. of the vapour would be absorbed. Prolongation of the time of exposure to the vapour would favour further dissociation, so that on theoretical considerations, it would seem impossible to produce acute poisoning in an animal with cobalt carbonyl vapour.

In order to test the accuracy of these considerations, rabbits were placed in the glass chamber and allowed to breathe the air mixed with cobalt carbonyl vapour. The temperature of the water jacket was varied in different experiments between 20° and 26° C. The rate of air current was also varied from 0·3 to 2·25 litres per minute. In one experiment, a rabbit was exposed for one hour to the vapour mixture. The cobalt carbonyl was volatilised at 21·5° C. and the air current was kept at 0·3 litre per minute, in order that saturation might be obtained. The temperature of the glass chamber rose from 14·3° to 16° C. during the experiment. After the experiment was over, crystals of cobalt carbonyl were collected from the tube beyond the water jacket, indicating that the air was saturated in the cooler portions of the apparatus. Only 0·175 mgr. of cobalt was recovered from the

bromine flask, while 1·2 mgrs. was recovered from the cotton wool plugs. In this case it was impossible to estimate the quantity of cobalt deposited in the chamber itself, as the rabbit's fur would offer as much surface for deposition as the glass walls. On the basis of the blank experiments, it could be estimated that the rabbit had absorbed not more than 0·14 mgr. of cobalt. As was expected, no symptoms of any description followed. Other experiments of a similar nature also yielded entirely negative results.

A rabbit was enclosed in a wooden box provided with glass windows in which was fixed a stretched piece of linen on which crystals of cobalt carbonyl were spread. The rabbit was allowed to remain in the box for two hours. The temperature inside the box rose from 13·5° to 18° C. A slight alteration in the colour of the visible mucous membranes indicated that some carbon monoxide had been absorbed. The amount of this was small. No symptoms of any kind were noted.

From these experiments the conclusion appears justified that owing to its low vapour tension and ready dissociability, cobalt carbonyl, contrasted with nickel and iron carbonyl, is unlikely to produce acute poisoning.

## PERIODICAL PUBLICATIONS.

*Bulletin of the Department of State Medicine and Public Health, Western Australia* (Nos. 7-9, July-September, 1908). 22 pp. Perth: F. W. Simpson, Government Printer.

*Publications from the Laboratories of the Jefferson Medical College Hospital.* Vol. iv. Philadelphia, 1908. Contents: The Significance of Tubercl Bacilli in the Faeces, by R. C. Rosenberger.—The Present Status of the Aetiology of Syphilis, by R. C. Rosenberger.—Aetiology of Cholelithiasis; Bacteriological Study of One Hundred and Two Calculi, by J. Funke.—The Pathological Anatomy of Bronchial Asthma, by A. G. Ellis.—Thrombosis of the Superior Mesenteric Artery with Hemorrhagic Infarction and Perforation of the Ileum, by J. Funke.—Some Unusual and Interesting Muscle Anomalies, by H. E. Radasch.—Lipoma of Uterus, by A. G. Ellis.—Accessory Pancreas, with Report of Two Cases, by A. G. Ellis.—Test to Determine the Adaptability of the Donor's Blood to the Receiver's Blood in Transfusion, by J. Funke.—A Study of the Volume and Specific Gravity of Organs, by S. L. Olsho.—Bacteriological Studies of Smegma, by R. C. Rosenberger.—Noma: A Bacteriologic Study of Seven Cases, by R. C. Rosenberger.—Purulent Cerebrospinal Meningitis caused by the Typhoid Bacillus without the Usual Intestinal Lesions of Typhoid Fever, by J. N. Henry, and R. C. Rosenberger.—Recent Literature of the Parathyroid Glands, by A. G. Ellis.—A Study of the Eosinophilic Cell as Occurring in the Hematopoietic Organs in Diphtheria and Tuberculosis, by G. B. Foster.—Congenital Unilateral Absence of the Urogenital System: Its Relation to the Development of the Wolffian and Muellerian Ducts, by H. E. Radasch.—The Blood Pressure in Tuberculosis, by W. B. Stanton.—Anencephalus, by C. H. Lefcowitch, and H. E. Radasch.—The Localization of the Motor Area Based on Exact Faradization, by A. Gordon.—The Pathogenesis of Reflexes, Apropos of a Case of Tuberculoma of the Spinal Meninges, by A. Gordon.—The Function of the Prefrontal Lobes, Apropos of the Pathologic Findings in a Case, by A. Gordon.

## REPRINTS.

DELÉPINE, S. (1908). The Essentials of Disinfection and Sterilisation by Steam at High and Low Pressures. *Medical Chronicle*, xii, 1908, 24 pp., 2 figs.

GANDY, Dr (1908). *Stations Thermales et Désinfection*. Toulouse: Imprimerie Ouvrière, 53, Rue Bayard, 53. 39 pp.

MILLER, W. W. (1908). Hepatozoon perniciosum (n.g., h.sp.); a Haemogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host (a mite: *Lelaps echidninus*). *Pub. Health and Mar.-Hosp. Serv., Treasury Dep't Hygienic Laboratory, Bull.* No. 46. 51 pp., 20 plates.

SOME INVESTIGATIONS ON THE TOXICOLOGY OF TIN,  
WITH SPECIAL REFERENCE TO THE METALLIC  
CONTAMINATION OF CANNED FOODS.

By S. B. SCHRYVER.

In July 1906 attention was drawn to the fact that a large number of tinned foods returned from the South African campaign were exposed for sale on the home markets. The majority of these foods were known to have been from five to seven years in tins, and the possession of this material afforded a rare opportunity for investigating the question of metallic contamination, and for determining how far such contamination was deleterious to the public health.

The following account of investigations into the subject is based on a report to the Local Government Board by Dr G. S. Buchanan and myself (Medical Department: Food Reports, No. 7, 1908) extracts from that report being reproduced with the permission of the Controller of H. M. Stationery Office.

Previous researches dealing with this subject have been published by Ungar and Bodländer (1887), working in Binz's laboratory, and by Lehmann (1902). The former investigators shewed that by repeated subcutaneous injections into animals of small quantities of tin in the form of a non-irritant organic salt (the double tartrate of tin and sodium) over prolonged periods, definite toxic symptoms could be produced, which resulted, after sufficiently long treatment with the metallic salt, in the death of the animal. The general effects of the poison were manifested (*a*) in disturbances in the alimentary tract, (*b*) in the general nutrition, and above all (*c*) in the central nervous system. In the case of rabbits and dogs the disturbances in the alimentary tract were only manifested when relatively large quantities of the salt were injected in the earlier stages of the experiments. In all cases repeated injections caused a marked diminution of weight and general wasting,

in spite of the fact that the animals continued to eat well till near the end, especially when the doses of tin salts injected were small. In most cases the wasting was accompanied by anaemia, probably due to the destructive action of the tin salts on the blood corpuscles (Löwenthal, 1902). The most marked changes were produced, however, in the nervous system, and were manifested in the first instance in the general disturbance of the power of motion in the hind limbs resulting finally in complete paralysis with anaesthesia. The animals at the same time exhibited a general depression of higher mental functions and intelligence. The general symptoms described by Ungar and Bodländer indicate not only disturbances of the central nervous system but also peripheral neuritis. They concluded that very small quantities of tin, when absorbed into the system, could cause serious disturbances to health, which could lead finally to a fatal termination. In the case of dogs, a daily subcutaneous dose equivalent to 6·75 mg. of metal per kilo of body weight resulted in the death of the animal after 8 days. With doses equivalent to 1·71 and 1·33 mg. per kilo of body weight, death resulted in 47 and 131 days respectively. These doses would correspond to daily doses of 6·2, 1·57 and 1·2 grains for individuals of 60 kilos weight.

Lehmann continued these investigations with the object of determining whether quantities of tin equivalent to those which could be ingested by the consumption of heavily contaminated canned foods were likely to result in tin poisoning with symptoms like those described by Ungar and Bodländer. He calculated that the maximum amount of tin likely to be ingested by a full grown man of 75 kilos weight, was 420 mg. (about 6·5 grains), the equivalent of 5·6 mg. per kilo. He experimented with cats, giving the animals with their food tin in doses gradually increasing from 5 to 40 mg. daily. The animals after prolonged periods of ingestion remained in perfectly normal health and increased in weight, and Lehmann concluded from the results of his experiments that the possibility of chronic tin poisoning from the consumption of canned foods is extremely remote.

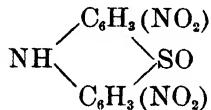
Apart from the question of chronic poisoning, it is necessary to consider the irritant action on the alimentary tract which can be produced by the ingestion of relatively large quantities of tin salts, especially when the latter are, as tin chloride is, of somewhat corrosive nature. Records of severe disturbances to the alimentary tract following the ingestion of canned foods are to be found in the literature, of which the best authenticated cases are described by Ungar and Bodländer (1887), Luff and Metcalfe (1890) and Günther (1899). In all

these cases estimations of the metallic contaminations of the food-stuffs were made. Ungar and Bodländer found that various tins of the canned asparagus to which the illness was ascribed contained from 2·1 to 2·8 grains per pound (300 to 400 mg. per kilo). In Luff and Metcalfe's cases, canned cherries contained no less than 24 grains to the pound (3430 mg. per kilo), whereas in Günther's case pickled herrings preserved in vinegar contained 7·2 grains per pound (1030 mg. per kilo) in the solid, and 2·2 grains per pound (316 mg. per kilo) in the liquid contents of the tin. The general results of previous investigators tend to shew therefore, that whilst chronic tin poisoning is unlikely to result from ingestion of canned foods, the possibility of irritant action cannot be entirely excluded. These conclusions are in the main confirmed in the present inquiry, where direct experimentation has been possible.

*The detection and estimation of tin in food-stuffs and tissues.*

Investigations were carried out as to the best methods for determining the amount of tin in food-stuffs and tissues and two were finally adopted, viz. a colorimetric method, specially adapted for estimation of small quantities, and a gravimetric method, which was chiefly employed when relatively large quantities of the metal were present.

*Colorimetric method.* The reagent used for the colorimetric determination was dinitrodiphenylaminesulphoxide



which was originally prepared by Berndsen (*Liebigs Annalen*, 1885, ccxxx. 116) by the action of nitric acid on thiadiphenylamine.

This substance on treatment with stannous salts is reduced to the corresponding diamino-derivative, which is the leuko-base of a dye-stuff, known from the name of its original discoverer as Lauth's violet. For the purposes of the analyses, 10 grams of the food-stuff or tissue are introduced into a 700 c.c. round-bottomed Jena flask together with about 10 grams of potassium sulphate; 10 c.c. of concentrated sulphuric acid are then introduced together with some water. The whole is then gently heated, and the diluted sulphuric acid first hydrolyses the proteins into simpler substances, which are more readily destroyed by the acid than the original protein. After the mixture has charred and

frothed up, more sulphuric acid is added, and the heating is continued till the whole of the organic matter has been destroyed. The amount of acid required for destruction will depend upon the nature of the tissue or food-stuff, and the amount of water it contains; 20 c.c. were in most cases sufficient, but in exceptional cases such as those of jams and meat extracts, containing large quantities of solid matter, as much as 40 c.c. were found necessary. After destruction of organic matter and cooling the contents of the Kjeldahl flask were diluted to 100 c.c., the liquid was saturated with sulphuretted hydrogen, and allowed to stand in a corked flask over night. The precipitated sulphur and sulphides were then filtered off on to a small filter paper (4 cm. diameter) and washed. The paper containing the precipitate was then transferred to a test-tube and boiled with 5 c.c. concentrated hydrochloric acid. After solution of the sulphide, the liquid was filtered through a Buchner funnel by means of a pump into a wide-mouthed test-tube with side-tube near the top by means of which it was connected with the pump. The filter paper remaining on the funnel was sucked as dry as possible, and washed with 2½ c.c. of strong hydrochloric acid, the washings being added to the filtrate. The test-tube was then connected with an apparatus for generating carbon dioxide, and the gas was led by a tube passing through a bored cork in the test-tube just over the surface of the liquid, the side-tube serving for its exit. A strip of zinc foil 2 inches long and  $\frac{1}{2}$  inch wide, and weighing about  $\frac{3}{4}$  gram was introduced into the liquid while still hot, and the stannic chloride was thereby reduced to stannous salt in the presence of a current of carbonic acid gas. As soon as the *last traces* of the zinc had dissolved, the reagent was added. This was made by dissolving 0·2 gram of dinitrodiphenyl-amine sulphoxide in 100 c.c. of  $\frac{N}{10}$  sodium hydroxide and filtering. 2 c.c. were employed for each test, the cork being momentarily removed from the test-tube for its introduction, and the carbon dioxide being continually passed the whole time. The nitro-body was precipitated at first, but redissolved on further warming. After heating for one or two minutes the mixture was diluted to 100 c.c. with cold water, one or two drops of ferric chloride solution were added, and the mixture was filtered. In the presence of tin a violet colour appeared, the intensity of which served as a measure of the amount of tin present in the substance analysed. The whole process, after the filtration of the sulphide, only takes a few minutes, and as a rule several analyses were carried out simultaneously. For determining colorimetrically the amount

of tin, a standard solution containing 14.28 mg. in each 5 c.c. was prepared. This was diluted with 5 c.c. of water, and 1 c.c. of the diluted solution contained therefore 1.428 mg. This quantity added to 10 grams of food-stuff was equivalent to a contamination of 1 grain per pound. For preparation of standard colours, 1 c.c., 0.75 c.c., 0.5 c.c. and 0.25 c.c. respectively, of the diluted solution were added to 7.5 c.c. of concentrated acid. Zinc (of standard size as above) was added, and the stannic salt reduced to stannous salt in a current of carbon dioxide and the reaction carried out with the dinitrodiphenylaminesulphoxide reagent in the way already described. A series of standard colours could thus be prepared, to serve as a comparison for those produced by the tin-contaminated food-stuffs or tissues. It must be remembered that the depth of colour is not in proportion to the amount of tin; the reaction is less complete in the more dilute experiments and the influence of mass action must be taken into account. The colour given by quantities of tin equivalent to 1 grain per pound (1:7000) is deep purple, and it is necessary to carry out the determination with diluted aliquot parts of the original solution when the contamination exceeds this amount. The colour is quite marked with quantities equivalent to  $\frac{1}{4}$  grain per pound.

*Gravimetric estimation.* For the purposes of gravimetric estimation, the organic matter was destroyed by heating with a mixture of sulphuric acid and potassium sulphate. After solution, the tin was precipitated by sulphuretted hydrogen as sulphide, which was filtered off, washed, oxidised in the usual way, and finally weighed as  $\text{SnO}_2$ . With samples containing more than 1 grain of tin, 50 grams of material were sufficient for each analysis, for those containing smaller quantities the colorimetric method was preferable. The latter was, however, always employed as a matter of routine for sorting purposes.

#### *The contamination of foods by tin.*

A large number of estimations of tin in different kinds of food-stuffs were carried out, of which the full details are given in the original report. The flesh foods from South Africa, of the approximate age of 6—8 years, contained quantities of tin varying from 0.36 to 2.06 grain per pound (51 to 294 mg. per kilo) the higher quantity having been found in a sample of tripe. The majority did not contain, however, more than 1 grain per pound. Meat essences, extracts and soups contained, however, larger quantities, the contamination being generally

more than 1·5 grains per pound, reaching in one case to more than 5 grains per pound. In several cases the contamination was 3·5 grains per pound, and in one tin of meat essences into which the solder had dropped the contents contained more than 21 grains per pound. Puddings and jams were also somewhat heavily contaminated, the quantities of tin ranging from 1·42 up to 5·13 grains per pound (203 to 733 mg. per kilo). It is conceivable therefore that in certain cases injurious results can accrue from ingestion of old canned foods. In addition to analyses carried out for the purposes of the report, data referring to tin contamination of foods were found in the literature and a numerical summary of the results obtained by different observers and by myself is given in the accompanying table. The summary has been made as a convenient way of exhibiting the results of some 130 analyses of different canned foods (exclusive of cans which had been punctured, or those in which obvious contamination by solder had taken place). It should be remembered, however, that although most of the foods in question have been "old," the periods of keeping are very varied and the results have been obtained by different methods.

	No.	Percentage of total
Food-stuffs containing less than 1 grain per pound	72	55·4
" " between 1 and 2 grains per pound	35	26·9
" " 2 and 3 " "	17	13·0
" " more than 3 " "	6	4·6

#### *Physiological experiments.*

The animal experiments of Lehmann (1902) render it improbable that tin, when ingested in quantities such as are found in contaminated canned foods, can cause symptoms of chronic poisoning. The experiments recorded in this section tend to confirm Lehmann's conclusions, and to shew that by far the largest quantity of the metal ingested *per os* is excreted without leaving the alimentary tract.

The first experiment was carried out on the person of the author of this communication, who at the time was in normal health and weighed 65 kilos. The metal was ingested in the form of the non-irritant double tartrate of tin and sodium, which was taken continually for a period of three weeks. During the first week the daily quantity ingested was approximately 1 grain (64·5 mg.), during the second week two grains (129 mg.) and during the third week 3 grains (193·5 mg.). The intake commenced on a Saturday at mid-day meal and was continued

throughout the week till breakfast on the following Saturday. Four meals were taken each day and a quarter of the daily dose was taken at each meal. This during the first week amounted to  $\frac{1}{4}$  grain. On Saturday at luncheon after the first week the dose was increased to  $\frac{1}{2}$  grain, and on the following Saturday at the corresponding meal to  $\frac{2}{3}$  grain. From Monday morning at 9.30 to the corresponding time on the following Saturday in each week, the total excreta, both urine and faeces, were collected; the quantities collected in each 24 hours were kept separately and separately analysed. No standard diet was adopted, although an effort was made to live as regularly as possible. In the faeces the total nitrogen and tin were estimated and in the urine the total nitrogen, the ammonia, the urea, uric acid, and the tin.

The output of tin during three successive periods of five days was thus determined and compared with the intake. It must be remembered that the main object of this experiment was to determine whether the excretion of tin kept pace with the intake, and for this reason, the method of analysing the excretions during a definite period in each week was purposely chosen. The method is, however, not without sources of error, the chief of which is due to the fact that it is impossible to get perfectly regular defaecation, in which case the faeces excreted during a certain period would not correspond with the material ingested. It was thought, however, that with periods as long as five days, this source of error would not be very great. The total nitrogen of the faeces and urine were, however, both determined, and it was found that the ratio of the nitrogen excreted in the urine to that excreted in the faeces did not vary greatly from week to week, and these small variations, furthermore, bore no relation to the proportion of tin in the excretions. Some of the chief results are indicated in the following table.

#### *Urine Analyses.*

	Total nitrogen excreted (grams)	Total tin excreted (grams)	Total tin ingested during 5 days (grams)
Five days of first week April 23rd—27th	76.9	0	.3300
Five days of second week April 30th—May 4th	72.2	.0550	.6600
Five days of third week May 7th—May 11th	70.8	.0763	.9900

The analyses of urea, ammonia, etc., indicated that no disturbances of metabolism had occurred during the period of tin ingestion.

*Analyses of Faeces.*

Date	Weight of faeces excreted (grams)	Per cent. N.	Total N. (grams)	Per cent. Sn.	Total Sn. (grams)	Tin Ingested
April 23	95	1·31	1·24	.0383	.0364	Approximately 1 grain per day.
,, 24	121	1·68	2·03	.0693	.0838	
,, 25	150·5	1·36	2·04	.0511	.0769	
,, 26	58·7	1·90	1·11	.0746	.0438	
,, 27	139	1·84	2·55	.0733	.1019	
Total 5 days	564·2	1·59	8·97	.0607	.3428	.3300
April 30	132	1·91	2·52	.1123	.1482	Approximately 2 grains per day.
May 1	69	1·96	1·35	.1397	.0964	
,, 2	91	1·92	1·73	.1251	.1138	
,, 3	73·5	1·95	1·41	.1209	.0888	
,, 4	156	1·67	2·60	.1209	.1885	
Total 5 days	521·5	1·84	9·61	.1218	.6357	.6600
May 7	89	1·36	1·21	.1470	.1308	Approximately 3 grains per day.
,, 8	141	1·69	2·38	.1740	.2453	
,, 9	112	1·73	1·93	.1354	.1516	
,, 10	52	1·80	0·94	.1846	.0966	
,, 11	105	1·87	1·96	.1797	.1886	
Total 5 days	499	1·69	8·42	.1628	.8123	.9900

The following table gives the ratio between the nitrogen excreted in the urine and faeces.

	Total Nitrogen ingested calculated from N. in urine and N. in faeces (grams)	N. in urine (grams)	N. in faeces (grams)	Physiological food value	Percentage unutilized N.
First week	85·9	76·9	9·0	89·5	10·5
Second week	81·8	72·2	9·6	88·3	11·7
Third week	79·2	70·8	8·4	89·4	10·6

The following table summarises the results and indicates the paths of excretion of the metal.

	Tin ingested (grams)	Total tin excreted (grams)	Total in urine	Total in faeces	Percentage of total tin recovered which was	
					Present in urine Per cent.	Present in faeces Per cent.
First week	.3300	.3428	.0	.3428	.0	100
Second week	.6600	.6907	.0550	.6357	7·9	92·1
Third week	.9900	.8886	.0763	.8123	8·6	91·4

From the above results it is evident, that when the quantities of tin ingested were one or two grains per day, no evidence of accumulation was forthcoming at the end of a fortnight. The quantities excreted are substantially the same as those ingested, the differences lying well

within the limits of experimental error. In the first week the quantities of tin excreted in the urine were so minute, that it is difficult to state with certainty that even traces were present. In the second week there was distinct evidence that some of the metal had been absorbed from the alimentary tract, and the amount excreted in the urine amounted to 8 per cent. of the total. When the quantity of tin ingested reached the amount of 3 grains daily, however, the output failed to keep pace with the intake, the deficiency in the excretion amounting to 10·3 per cent., a larger quantity than could be accounted for by any irregularity in the defaecation, as reference to the table shews that the ratio of nitrogen excreted in the faeces to the total nitrogen ingested was the same as that in the first week, when the tin ingested was quantitatively recovered in the faeces.

Similar experiments were carried out with a dog weighing 6·7 kilos, which received with its food during the first week 10 mg. daily, during the second week 20 mg. daily and during the third week 30 mg. daily. Practically the whole of the tin was recovered in the faeces.

The above experiments indicate at first sight, that of tin ingested *per os* comparatively little leaves the alimentary tract. Ungar and Bodländer have shewn, however, that when tin is subcutaneously injected, part leaves the system in the faeces. It is therefore possible that some of the tin excreted in the faeces in my experiment, had been absorbed before excretion into the gut. The following experiments indicate, however, that when the metal has been once absorbed into the system, the great part leaves it again in the urine, and that, therefore, there is but small absorption from the alimentary tract.

A medium-sized dog (about 7 kilos) received daily a subcutaneous injection of five milligrams of tin in the form of a double tartrate for six days. 127 grams of faeces (dried weight) were collected and also the whole of the urine excreted during the period of experiment and the three subsequent days. A quarter of the total of excreta were submitted to colorimetric analysis for tin, and it was found that a quarter of the urine contained about 1·5 mg. of the metal, i.e. 6 mg. of the total of 30 mg. were found in the urine. The faeces contained only about two-thirds of this quantity.

A second experiment with another animal, which received 20 mg. of metal subcutaneously, lead to a similar result. In both these cases small doses of the metal only were injected. In the following experiment relatively large quantities of tin were injected within a short interval. In this case, a considerable proportion is rapidly excreted by the urine.

A dog of 8·5 kilos weight, one hour and a half after an injection of morphia, was fully anaesthetised with A.C.E. mixture. Cannulae were then inserted in Wharton's duct and the ureters, and a manometer was connected with the femoral artery. 100 milligrams of tin in the form of the double tartrate dissolved in 100 c.c. of warm physiological saline were then injected into the external jugular vein. No change in the blood pressure was caused thereby. During the course of the next hour and a half, 34 c.c. of urine were obtained and 10 c.c. of saliva, the latter being produced by electrical stimulation from time to time of the corda tympani. Throughout the whole experiment the animal was kept in a state of complete anaesthesia, and it was killed by a heart incision whilst still under the influence of the anaesthetic. The saliva, urine, and various organs of the body were then submitted to colorimetric analysis for the purpose of estimating approximately the distribution of tin.

The mucous membrane of the large intestine weighed 8 grams, of which 4 grams were used for analysis. Tin was found to be absent.

The mucous membrane of the small intestine weighed 93 grams, of which 25 grams were used for analysis. In this quantity were found about 0·5 mg. Sn.

The liver weighed 218 grams, of which 25 grams were used for analysis. In this quantity were found about 2 mg. Sn, indicating therefore a very appreciable amount in the whole of the liver.

The central nervous system (brain and spinal cord together) weighed 65 grams, of which 20 grams were used for analysis. This quantity was found to contain 1·5 mg. Sn.

Tin was absent from the saliva.

34 c.c. of urine were found to contain no less than 18·4 mg. Sn, which was estimated gravimetrically in 17 c.c. of the liquid.

#### CONCLUSIONS.

The general content of the above work may be briefly summarised as follows:

1. A method is described by means of which small quantities of tin in food-stuffs and tissues can be rapidly estimated colorimetrically.
2. A summary is given of a large number of analyses of canned foods contaminated by metal, most of which had been in tins for several years.
3. As a result of the experiment by Lehmann on animals, and an

experiment described above on a human being, it is concluded that there is but little likelihood of chronic tin poisoning resulting from ingestion of canned foods.

4. Nevertheless, cases have been recorded in the literature, describing symptoms of irritant poisoning following the ingestion of contaminated foods, and in certain of these cases the amount of metallic contamination has been ascertained.

5. It is difficult to state the exact quantity of tin salts which will give rise to symptoms of irritant poisoning, and these toxic effects will vary greatly with circumstances. Quantities of tin approximating to two grains to the pound are, however, unusual and unnecessary, and any food-stuffs containing such quantities should be regarded with suspicion.

#### REFERENCES.

- GÜNTHER (1899). *Zeitschr. f. Untersuchung v. Nahr. u. Genussmittel*, II. 917.  
LEHMANN (1902). *Archiv f. Hygiene*, XLV. 88.  
LÖWENTHAL (1902). *Deutsche med. Wochenschr.* p. 254.  
LUFF and METCALFE (1890). *British Med. Journ.*, I. 833.  
UNGAR and BODLÄNDER (1887). *Zeitschr. f. Hygiene*, II. 241.

## THE PROGRESS OF ANKYLOSTOMIASIS IN CORNWALL.

By A. E. BOYCOTT AND J. S. HALDANE.

(*From the Gordon Pathological Laboratory, Guy's Hospital.*)

ANKYLOSTOMIASIS was first discovered in men working in Cornish mines in October, 1902 (this *Journal*, Vol. III. p. 95), though there had been many cases of illness clearly referable to this cause for some eight years previously. The number of men who were too ill to do their ordinary work was greater about two years before the cause of "Dolcoath anaemia" was identified. These men had either stopped at home or had been employed on the surface instead of underground: in this way they had avoided further infections, and in many cases had progressed towards a spontaneous cure. At the time therefore of our first enquiry the number of sick men was less than it had been. Anaemic miners were, however, still very numerous and, without anything like an exhaustive search among the 750 underground hands at Dolcoath mine, we found 14 men with less than 50 p.c. haemoglobin and 19 more with less than 75 p.c. The general pallor prevalent among the men on looking at a shift as a whole was very striking, and complaints of shortness of breath on climbing the ladders were frequent. The disease, in short, was at that time a material hindrance in carrying on the work of the mine.

Examination of the faeces and blood of men who complained of no symptoms of ankylostomiasis showed that practically every underground worker was infected in Dolcoath. In December, 1903, and February, 1904, 65 men taken at random were examined by the "blood test"<sup>1</sup> and 61 (94 p.c.) found infected. The sanitary circumstances of the mine were

<sup>1</sup> This *Journal*, Vol. IV. 1904, p. 437.

at this time extremely unsatisfactory: there were no special arrangements for the reception of excreta underground and the whole of the workings were extensively soiled with faeces. The wetness and warmth of the deeper workings and the upcast shafts contributed to make Dolcoath an ideal home for *Ankylostoma*.

Industrial efficiency was obviously confronted here with a problem of some importance. The steps taken to escape the consequences of ankylostomiasis were simple, practical and apparently efficacious. Once the nature of the disease was recognised it was, in the great majority of cases, very easy to cure those actually sick by the repeated administration of appropriate anthelmintics: in the case of Dolcoath thymol has been exclusively used. The effects of this treatment have been almost entirely gauged by the clinical results: no serious attempt has been made to ascertain whether all the worms had been killed in any particular case if the man's general condition improved so far that he was able to return to work. Having been restored to a condition of efficiency, therefore, the men returned to work in a place which was still infected. They were of course reinfected again and again; any return of symptoms was noted at once and corrected by a dose of thymol. In this way actual sickness from ankylostomiasis has been practically abolished. At the same time steps were taken to introduce sanitary reforms into the underground workings. In 1905 a Special Rule was established by the Secretary of State for metalliferous mines in Devon and Cornwall that:

"The owner, agent or manager shall cause a sufficient number of suitable sanitary conveniences to be provided above and below ground in suitable and convenient places for the use of the persons employed, and to be constantly kept in a clean and sanitary condition, and no person shall relieve his bowels below ground elsewhere than in those conveniences. No person shall soil or render unfit for use in any way any convenience or sanitary utensil or appliance provided for the use of the persons employed."

In 1904 such sanitary appliances were actually in use in Dolcoath in the form of loose buckets or pails<sup>1</sup> which could easily be brought to surface, emptied and cleaned.

In April, 1908, we made, on behalf of the Royal Commission on Mines, further enquiries at Dolcoath and other Cornish mines to see what

<sup>1</sup> See Report to the Home Secretary on the Health of Cornish Miners, by J. S. Haldane, J. S. Martin and R. A. Thomas, Cd. 2091, 1904, p. 31 and figure 6.

effect these measures had produced. The conditions underground had been immensely improved, the pails were properly used and we were told that it was now very uncommon for the men to fail to use them. Personally we could find no faeces in a short search in places where, as previous experience had shown us, they would be most probably found. The general appearance of the men was quite different, and there was none of the general anaemia which had been so obvious five years before. On carefully going through two-thirds of the underground hands, we found only one case of definite anaemia in the person of a boy. There was only one man away from work with symptoms which could possibly be due to ankylostomiasis; he was not available for examination. In making a detailed examination of individuals by the "blood-test" we examined especially those who had worked at Dolcoath for periods varying from four months to three years, *i.e.* who had come to the mine since the introduction of underground pails—and who had not previously worked underground or only in such mines as were known to be free from *Ankylostoma*. Of 89 persons, mostly boys, who came in this category, 68 (76 p.c.) were found to be infected, and most of them gave a history of having had "bunches" on one or more occasions. The important conclusion follows from these results that the improved sanitary conditions underground had put an end to material illness but had had hardly any effect in diminishing the number of men infected. The whole improvement cannot be attributed to the accurate diagnosis and prompt treatment which now prevail. The management of the mine are quite clear that there has been a very large reduction in the number of cases of slight illness; and the men themselves say that "bunches" occur less frequently and less severely. The sanitary pail system, therefore, has evidently had a great effect in reducing the infectivity of the mine. That three quarters of the new hands still become infected soon after beginning work in Dolcoath shows however that the larvae must still be pretty widely spread in the mine. If however there were as many larvae as before there would be more cases of incipient ankylostomiasis. The reduction in actual illness confirms the view that as a rule severe illness is only brought about by massive infection.

The essential result of the simple measures taken is that *Ankylostoma* no longer causes any industrial inconvenience in Dolcoath. This result has been brought about without any expense or special organisation beyond the provision and care of the sanitary pails. The alternative method of treatment would have been to try and stamp out the infection altogether both in the men and in the mine, and to prevent its importa-

tion in the future by a rigorous medical examination before allowing any fresh hands to work underground. This method has been adopted in Westphalia and more or less in Belgium. Applied to mines which were in the first instance not nearly so heavily infected as Dolcoath, the method has so far failed to stamp out infection entirely. Every man infected with *Ankylostoma*, as well as the small proportion of them suffering from ankylostomiasis, is searched out, kept away from work (on compensation wages) and treated until repeated examinations fail to show any eggs in his faeces<sup>1</sup>. He is then allowed to return to underground work: the workings are of course still infective, so he becomes reinfected and the whole process has to be gone through again and again. After several years of this extremely troublesome and expensive procedure, a few men are still found to be infected. If the periodical examination and treatment of the men were now relaxed, there is no guarantee that in a short time the infection would not spread from these few remaining carriers and again become prevalent. The failure of this attempt may be in part due to the impossibility of making sure that every man who is treated is really quite free from worms. It is however doubtless more dependent on the longevity of the adult larvae and the difficulty of attacking the infection of the mine. We have found that the encapsulated larvae will live in the laboratory for more than a year. Their resistance to disinfectants has been much exaggerated (this *Journal*, IV. 1904, p. 85), but there are obvious difficulties in the way of efficiently permeating all the mud and water, along perhaps miles of underground roads, with any antiseptic in sufficient concentration to kill them. It is however a very long business to disinfect the men and wait for the larvae to die out. If the eradication of the parasitic phase of the worm is to be effective, it is necessary that its saprophytic phase should be also attacked. That this is possible, though necessarily troublesome and expensive, is, we think, indicated by the history of Levant Mine (see this *Journal*, IV. 1904, p. 447). *Ankylostoma* has never gained any foothold in this mine, where the circumstances of temperature, moisture, faecal contamination and the presence of infected persons are all very favourable for its spread. The immunity is certainly due to the fact that sea water leaks into the mine so that the salinity of the mine water varies, in different places, from 0·9 to 3·0 p.c. Our observation that less than 2 p.c. sodium chloride will kill young *Ankylostoma* larvae in the laboratory has been confirmed by Calmette<sup>2</sup>; and the fact that

<sup>1</sup> The simple blood examination is of course not applicable under these circumstances.

<sup>2</sup> *Lancet*, Vol. III. 1905, p. 490.

the infection does not gain any hold in mines with saline waters has subsequently been fully established by Manouvriez<sup>1</sup> and Tirelli<sup>2</sup>. If any method of disinfection is likely to be successful, it seems to us that chief attention might be paid to salt which has naturally proved so efficacious.

As far then as the infected mine itself is concerned, it does not seem to us that the benefits which have been gained in Westphalia are greater than those in Cornwall to a degree at all commensurate with the enormous sums of money which have been spent on medical examination and treatment and the payment of wages to men under treatment. But the German system has this great advantage—that it has, within a few years, very largely reduced the number of men who were capable of carrying the infection to fresh places. This result could not be obtained by the Cornish system except after a very long time. Any dozen men from Dolcoath are at the present time not much less likely to infect any suitable mine to which they might go than they were five years ago, except in so far as their habits of defaecation have improved.

So far as this country is concerned, there seems to be no very great danger of serious infection becoming general among miners. In this matter the experience of the mines in the neighbourhood of Dolcoath is not without interest. There has always been a very free interchange of men between Dolcoath and these other mines. But even before the nature of the disease was recognised, there was much less illness in them, and the disease never caused the trouble that it did at Dolcoath. This is doubtless due to the fact that they are, on the whole, not so deep, cooler, and in some instances drier than Dolcoath. In 1903, 42 p.c. of the men at East Pool Mine were infected, and 12 p.c. of those at South Crofty, men who had worked at Dolcoath not being reckoned in each case. In 1908, 15 p.c. of the men at Grenville Mine who had never worked elsewhere and 24 p.c. of all the men were found to be infected : for Tincroft the corresponding figures were 9·5 p.c. and 25 p.c. At these last two mines 48 p.c. of the men who had previously worked at Dolcoath were found to be infected : in a number of cases they had not been in Dolcoath for several years. We could only find one instance of illness at these mines. These results show clearly that, despite very free interchange of infected persons, the disease may cause no

<sup>1</sup> *Mines rendues réfractaires à l'ankylostome par des eaux salées de filtration*, par A. Manouvriez, Valenciennes, 1905.

<sup>2</sup> *Lancet*, Vol. i. 1908, p. 102.

practical inconvenience in places which are moderately unsuitable for the development of the larvae.

It appears that there are in this country not many mines where the general conditions of temperature and moisture are suitable for *Ankylostoma*. Most of the hot mines are, except in limited areas, too dry, and most of the wet mines are too cool. Careful enquiries on this point were made recently by Prof. J. Cadman for the Mines Commission. He found very few hot wet mines. A number of the men working in those where favourable conditions prevailed in different parts of England and Scotland were examined by one of us (A. E. B.) by means of the blood film method<sup>1</sup>; no evidence of the presence of *Ankylostoma* was obtained, even in a pit where men from infected mines in Germany had been working. The realisation of the possibility of *Ankylostoma* gaining a foothold in this country has, however, been most beneficial in directing attention to the unsanitary conditions which often prevail underground, and in bringing about a much-needed reform in these matters. There is every reason to believe that when the reforms recommended by the Mines Commission are carried into effect the coal mines of this country will be proof against any appreciable *Ankylostoma* infection.

This enquiry showed once more the usefulness of the blood film method in searching for *Ankylostoma*. Since some misapprehension has arisen as to its scope and accuracy, we may take this opportunity of briefly stating the data which have been obtained. We ascertained in Cornwall in 1904 that of 148 men infected with *Ankylostoma*, 94 p.c. showed more than 8 p.c. of eosinophiles in a differential leucocyte count made on a dried blood film, while only 3·5 p.c. had less than 5 p.c. of eosinophile leucocytes. On the other hand, of 158 miners not infected with *Ankylostoma* in Cornwall, Staffordshire and Shropshire, 91 p.c. had less than 5 p.c. and only 2 p.c. more than 8 p.c. of this variety of leucocyte. In 1907-8 we examined films from 642 men in hot wet coal and metalliferous mines in Scotland, Lancashire, Yorkshire, South Wales and the Isle of Man: only four (0·6 p.c.) showed more than 8 p.c. eosinophiles. In 800 non-infected miners therefore we had seven cases of eosinophilia: in one the man had left the district and his faeces could not be obtained, in two *Ascaris* eggs were found, in the four others *Trichocephalus* only. An incredible amount of time would have been consumed in obtaining and examining samples of faeces from these 800 working men. As an illustration of the rapidity with which the blood method may be carried

<sup>1</sup> See *Second Report of the Royal Commission on Mines*, Parliamentary Paper, 1909, p. 182.

out we may note that 183 films were obtained by us from two pits in Scotland and examined in London in four days: one case with 15 p.c. eosinophiles was found and *Ankylostoma* was definitely excluded by an examination of his stools during the next week. In this country therefore the method works admirably. But this is so only because other intestinal worms which cause eosinophilia are quite uncommon. The ordinary hospital population in London only harbour worms to the extent of 5 p.c.; and practically all these are *Trichocephalus*, which very rarely produces a definite eosinophilia<sup>1</sup>. This method could not be applied with the same success to, for example, an ordinary Swiss population where *Ascaris*, a frequent cause of a high degree of eosinophilia, is found in 48 p.c. of people<sup>2</sup>: still less in the tropics where most natives have more than one cause of eosinophilia in their persons besides *Ankylostoma*. No suggestion has however ever been made by us that it should be used under these circumstances. Nor has it been proposed that the examination of blood films should replace the search for eggs in the faeces in persons in whom a *prima facie* case for the presence of *Ankylostoma* has been made out. The ultimate diagnosis, which is preliminary to treatment, must rest on the examination of the faeces; a preliminary examination of the blood will however save a great deal of trouble in cases, as in English miners, where other causes of eosinophilia are practically absent.

<sup>1</sup> French and Boycott, *This Journal*, Vol. v. (1905), p. 274.

<sup>2</sup> B. Galli-Valerio, *Centralbl. für Bakteriol. Orig.* Vol. xliiv. (1907), p. 531.

## ON THE NATURE OF THE CELLULAR ELEMENTS PRESENT IN MILK.

(FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.)

By R. TANNER HEWLETT, M.D., F.R.C.P., D.P.H.,

*Professor of General Pathology and Bacteriology,  
King's College, London,*

SIDNEY VILLAR, F.R.C.V.S.,

*Veterinary Inspector for the County of Middlesex,*

AND CECIL REVIS,

*Chief Chemist, Messrs Welford & Sons, Ltd.*

THAT cellular elements are found normally in milk has been known for many years, but it is only comparatively recently that any importance from a public health point of view has been attached to their presence. These cellular elements being usually regarded as leucocytes or pus cells, it is natural that their occurrence in milk should be looked on as indicative of an inflammatory process in the udder of the cow or cows from which the milk was obtained. The first observers to make any attempt to arrive at the numbers of these cells in milk seem to be Stokes and Wegeforth<sup>(1)</sup> in 1897. Their method, however, was very crude, and is not suited for making a true enumeration. Stewart<sup>(2)</sup> and Slack<sup>(3)</sup> followed with a very similar method, except that special tubes were used for obtaining the deposit. This "smeared sediment method" as it is called, is, however, capable of great inexactitude. Later observers have, by the use of ordinary blood counters, made the counting of the cells in milk a scientific measurement of reasonable accuracy. To Doane and Buckley<sup>(4)</sup> belongs the credit for the first published use of such a method. Savage<sup>(5)</sup> however has made the process still more

accurate by first diluting the milk before centrifugalisation, and also by counting all over the field of the blood chamber, two innovations which are of very great importance, as has been borne out by our investigations. Error is easily introduced if counting is confined to the ruled divisions of the counter. Recently, Trommsdorff<sup>(6)</sup> has used a method based on the measurement of the deposit obtained on rotation in a graduated tube, and this method, probably on account of its ease and speed, has found much favour in Germany. It has been severely criticised by several investigators, such as Schuppius<sup>(7)</sup> and one of us<sup>(8)</sup>. Rühm<sup>(9)</sup> and some others believe that it is useful if used as a routine control method. We are of opinion that it might be used in such a way as an "indicator," but for the purposes of public health control it is quite hopeless.

The outcome of the elaboration of methods for estimating the number of cells present in milk has been an attempt to diagnose mastitis amongst the cattle supplying any particular milk. For this reason milk has been examined by many observers for the purpose of ascertaining how many cells are normally present. The discrepancies among the results of the various investigators are to be attributed (1) to the different methods used; (2) to a want of appreciation of the sources of error even in the more refined methods; (3) to the use of isolated milks without reference to circumstances; and (4) to a want of careful enquiry into, and supervision of, the milks employed. The most reliable results are those obtained by Russell and Hoffmann<sup>(10)</sup> working with cows under strict supervision and over long periods, and these results have been fully confirmed by one of us<sup>(8)</sup> working under similar conditions. Quite recently Savage<sup>(8)</sup> has given a large number of results obtained from cows both in health and disease, and though the samples are, in many cases, isolated instances, still the conditions were accurately known. As a result of investigation, the possibility of setting a limit to the number of these cells for the purpose of public health control is seen to be very doubtful. A limit of 500,000 per c.c. seems to be tentatively held by many, though at the same time their own results show that much injustice would be done if such a limit were enforced, both by the condemnation of sound milks and by the passing of diseased milks. It has been part of this investigation to make weekly enumerations of the cells found in the milk of several herds under careful veterinary inspection, and as a result it has been possible to show that such a limit of 500,000 per c.c. would often condemn milks from quite healthy sources.

The results of Russell and Hoffmann are particularly interesting, in that they attempted to trace the influence of such factors as parturition and lactation, of feeding and temperature, etc., on the number of cells appearing in the milk without being able to detect any definite connection, and we also have arrived at similar conclusions.

Bergey and Savage have both made enumerations of the cells occurring at the same time in the milk from separate quarters of the udder, and they both find very great variations in the numbers so obtained. The importance of this fact in its bearing on the question of the nature of the cells found in milk is great, and does not seem to have been properly appreciated. The same importance attaches to the now well-known fact that cows which have had mastitis in one or more quarters show a large count of cells from those quarters long after the disease had subsided, and lactation has been re-established in the affected quarters.

So long, however, as this method of examination is confined to an enumeration of the cells only, no particular or special importance attaches to their precise nature, but quite recently there has been a tendency to diagnose the presence of "pus" in milk by a "qualitative" examination of the cells present. This has been done by assuming that the cells present are true leucocytes, and making a differential count in a manner similar to that employed in the case of blood. As such attempts have not been made merely for scientific interest, but have been used for the hygienic control of existing milk supplies, it becomes of paramount importance to ascertain beyond all doubt whether the nature of these cells justifies their classification as true leucocytes, and consequently their differential enumeration, as in the case of blood. In the course of an investigation into the Bacteriology of Garget, undertaken for the Local Government Board, Savage has made use of a classification into polymorpho-nuclear cells, lymphocytes, large leucocytes, and doubtful cells, but with his usual fairness, on account of discrepancies so found, he clearly states that the presence of any particular form of leucocyte cannot be taken as diagnostic of pus.

Before proceeding, however, to discuss the true nature of the cellular elements which come naturally into milk, it will be necessary to consider briefly some of the views which have been held with regard to the formation of milk in the udder, and the general functional activity of that organ. It is necessary clearly to realise the extraordinary secretive power of the udder, the secretion, both in its quantity and complex composition, being without parallel in any other secreting gland of the body.

Attempts have been made to elucidate this from as far back as 1838, when Donne first described the so-called "Colostrum granules," and from that time onward there has been no lack of investigators in this field. Without the least disparagement of the older workers, it is right that we should place more reliance on modern work, on account of the great improvements which have taken place in the technique both of preparation of tissues and of staining methods. Particularly is this of importance, as the real solution of the problem lies in the correct microscopical interpretation of sectional preparations of the udder during lactation.

Much confusion in such interpretations has undoubtedly arisen from a want of appreciation of the fact that the whole of the gland tissue is not in the same state of activity at the same time, so that in sections we shall expect to find some of the alveoli actively secreting, while others, having discharged their contents, are for the moment in a "resting" stage. The varying dimensions and form of the epithelial cells during extension of the alveolar space, and during contraction after expulsion of the contents, make it extremely difficult to recognise the cellular elements as the same in both cases.

As it is impossible to mention the views of the host of observers who have contributed to the solution of this problem, we shall briefly quote the excellent summary of such views made by Pfaundler. He distinguishes four typical glandular tissue conditions, which, however, are always closely connected, and are to be taken as occurring in succession in the actively secreting udder.

(a) The alveoli are open, the epithelial cells are cubical and contain clear round nuclei with one or two nucleoli. Mitosis is constantly present in the epithelium; the protoplasm of the cells shows fine granulation and vacuoles. The cells appear indefinite, their outline and limiting surfaces indistinct. There is a marked infiltration of the alveoli with leucocytes (many eosinophile), which are found in such great numbers in the interstitial tissue and the epithelial layer and lumina of the alveoli, that the remaining structure is only recognised with difficulty.

(b) The alveoli have very narrow lumina and the epithelium is cubical, the nuclei of which seem shrunken. Mitosis is constantly present. The protoplasm of the cells contains coarse granules and fat drops, and the cell outline is indistinct. The interstitial tissue is rich in leucocytes, but less so than in (a). In the lumen of the alveolus leucocytes, colloidal masses and colostrum bodies are present.

(c) The alveoli have very narrow lumina and long cylindrical or

pyramidal epithelial cells, clearly marked off from one another, some resting flat on the alveolar wall and some suspended by a narrow process. Each cell contains one to three nuclei, and at the free margin of the cell fat drops are often seen. Many of the cells which hang by a tongue of protoplasm into the alveolar space appear as if torn.

(d) The alveoli are dilated. The epithelial cells are flat, and in profile appear as ring-shaped narrow protoplasmic borders with spindle-shaped nuclei, and contain very few fat globules. Mitosis is rarely seen.

Particularly characteristic of this state are certain curious forms, first described by Nissen (*die Nisschenche Körperchen*). These bodies consist of nuclei with a characteristic arrangement of chromatin, partly still in the cells and particularly at their distal extremity, and partly in the lumen of the alveolus. The chromatin lies at the periphery of the nucleus in lenticular segmental masses, which ring round the chromatin-free inner nucleoplasm.

It will be noted in this summary that the infiltration of the interstitial tissue with leucocytes, and their extrusion into the lumen of the alveolus, seems to be generally held, but attention is particularly drawn to the pendulous epithelial cells under (c), and to the "Nisschenche Körperchen," to which reference will again be made.

It is now necessary to consider somewhat in detail the views of Winkler<sup>(11)</sup>. As the result of a careful histological investigation of the udders of cows during different periods of lactation and before parturition, and in cases of mastitis, he described the general structure of the alveolus as consisting of:

(a) The membrana propria, a structureless envelope and a cuticular formation of the interstitial connective tissue.

(b) A muscle fibre layer immediately within this, consisting of smooth muscle cells with very delicate elongated nuclei.

(c) The epithelial layer lining the lumen of the alveolus.

In these he is in general agreement with most other observers, but between (b) and (c) he describes a "germinal cell layer," which seems to have been noticed in 1877 by Kolissnikow, who pronounced it to consist of young epithelial cells, and also by Heidenhain in the submaxillary glands, who also describes it as consisting of small round often multi-nucleated epithelial cells. From this germinal cell layer Winkler shows that the epithelial cells of the gland are constantly renewed, and though normally the cells of the germinal layer lie behind those of the epithelial layer, still in sections (on account, perhaps, of the expansion

of the alveolus at the time of taking the tissue from the animal, and the subsequent contraction and distortion brought about by the fixing agent) the germinal cells and epithelial cells often appear to be in the same plane. In epithelial masses found during the colostral period the gradual transformation of these actively multiplying germinal cells into epithelial cells is easily observed. In some cases it would seem that the young germinal cells found in the germinal cell layer develop completely into epithelial cells, though in certain instances a division into an upper and under cell seems to occur, and the upper cell alone becomes an epithelial cell. In the case of the large multi-nucleated germinal cells, and also in young epithelial cells, there is often seen a growing out of one or more of the nuclei, accompanied with little cytoplasm forming a sort of horn which finally becomes separated from the parent cell, and passing through the epithelial layer is pushed out into the lumen of the alveolus. This peculiar action has given rise to an appearance in sections which has caused many to mistake this budding process for a wandering of leucocytes into the epithelium. In the extraordinarily marked nuclear multiplication in this germinal layer lies (according to Winkler) the solution of the much investigated question, "How do the epithelial cells multiply?" when multi-nucleated cells are seldom found in the epithelium, nor does mitosis often occur during lactation. The nuclei of these multi-nucleated cells are usually small, show no differentiation in their nucleoplasm, and so stain evenly and darkly, but in the developed epithelial cell the nucleoplasm exhibits the chromatin in sectors. The former cells also are more resistant and are not dissolved in the milk secretion, as the epithelial cells themselves often are when detached.

From the time of Rauber onwards the view seems to have been often held that leucocytes and lymphocytes are closely connected with milk formation. Rauber's original view that they are destroyed, and in their destruction are converted into the milk secretion itself, is now discarded, but many hold that during lactation there is a strong infiltration of the interstitial tissue by leucocytes, whence they pass through the epithelial cell-layer and enter the lumen of the alveolus. Winkler, however, maintains that the irregularly situated dark-staining nuclei seen in the epithelium are not those of leucocytes, but are the nuclei of "replacement cells," and this is true also of the early stages of mastitis before the secretion has become watery or bloody. The presence of fat globules in many of the cells found in milk clearly shows that these cells are epithelial, and not leucocytes. In these views he is in accord with Michaelis, who also maintains that leucocytes and lymphocytes play

no part in milk formation, and that if they do appear in large numbers it is a proof that suppuration is taking place or injury to the lymphatic vessels has occurred.

We may sum up the views of Winkler and Michaelis briefly by saying that, according to them, "the cells found in normal milk are chiefly young epithelial cells and cells of the germinal layer which have been detached, or thrust out into the lumen of the alveolus and so appear in the milk stream; that over activity of the germinal layer, and consequent increase in the number of these cells, may be the effect of change of food or some disturbance in milk formation, and is not indicative of disease; and, further, that in the early stages of mastitis it is the epithelial layer that is attacked by streptococci, and the destruction of this layer rouses the germinal layer into great activity, with a consequent increase of cells appearing in the secretion, but that no large number of leucocytes and lymphocytes are likely to be found until the mastitis is so far advanced that it shows itself by macroscopic changes in the milk itself; and finally that the multi-nucleated cells found in milk, and usually mistaken for polymorpho-nuclear leucocytes, are really detached young epithelial cells."

With these views we are in complete accord, and on account of the importance now being attached to the presence of multi-nucleated cells in milk, we have considered it advisable to bring these views forward at once, only appending a general statement of facts now in our possession in support of them, leaving the full statement of these until the work now in hand shall be completed.

This confirmation of Winkler's views has been largely brought about by an improvement in the method of staining these cells, which has enabled us clearly to demonstrate that in general structure, etc., these cells for the most part are clearly not leucocytes. That leucocytes do appear is evident from the fact that by this method of staining the presence of true eosinophile cells is easily demonstrated, but they are very few in number, and the vast majority of the cells tally in every particular with those described by Winkler.

Another important point hitherto apparently quite overlooked is that, in spite of the fact that these cells have often been for a considerable time in a liquid containing many bacteria, and under conditions in which normal leucocytes would retain their activity, phagocytosis is practically never seen, and this is true also when an invasion of the epithelium by streptococci has taken place, and destruction of its cells has followed.

We can briefly sum up those facts which support the view that the cells found in milk are for the most part not leucocytes as follows:

(1) The cells present in milk (the so-called leucocytes) are very diverse in nature, and when critically examined, the majority distinctly differ from leucocytes.

(2) However fresh the milk may be, the vast majority of the cells in it never stain like active leucocytes with ordinary blood stains.

(3) Though many multi-nucleated cells are present, the majority of these are distinctly different from polymorpho-nuclear leucocytes.

(4) The cells present in milk, however fresh, are scarcely ever amœboid.

(5) Ingestion of bacteria by the cells present (phagocytosis) is practically absent.

(6) In milk obtained from perfectly healthy cows these cells may occur in vast numbers, and since the mammary gland in structure resembles other glands, it is against analogy that vast numbers of leucocytes should occur in its secretion.

(7) The cause of the presence of a considerable number of cellular elements at times when there is no obvious reason, such as in quarters of the udder which have a previous history of mastitis, etc., but have recovered, is easily explained if these cells are tissue cells and not leucocytes.

#### REFERENCES.

- (1) STOKES and WEGEFARTH. *Journ. of State Med.* 1897, p. 439.
- (2) STEWART. *American Medicine*, 1905, No. 9.
- (3) SLACK. *Journ. Infect. Diseases*, 1906, Sup. No. 2.
- (4) DOANE and BUCKLEY. *Maryland Agricult. Exp. Station*, 1905, Bul. 102.
- (5) SAVAGE. *Journ. of Hygiene* 1906, p. 123.
- (6) TROMMSDORFF. *München med. Wochenschrift*, 1906, 12.
- (7) SCHUPPIUS. *Archiv für Hygiene* Bd. 62, H. 2, S. 137.
- (8) REVIS. *Journ. Roy. Inst. Pub. Health*, Dec. 1908.
- (9) RÜHM. *Wochenschrift für Tierheilkunde*, 1908, S. 125.
- (10) RUSSELL and HOFFMANN. *Journ. of Infect. Diseases*, 1907, Sup. 3.
- (11) WINKLER. *Zeitschrift für Landw. Versuchswesen in Oesterreich*, 1908, S. 562.

## BRITISH INDUSTRIAL ANTHRAX.

BY CECIL H. W. PAGE, M.D. CAMB.

### PART I.

(1 Figure.)

#### CONTENTS.

	PAGE
Introduction . . . . .	279
Historical . . . . .	280
Statistics . . . . .	281
History of Bristles and Horsehair in relation to Anthrax . . . . .	296
Manipulation of Bristles and Horsehair . . . . .	300
Dangerous processes and materials . . . . .	301
Anthrax among animals . . . . .	307
Summary . . . . .	312

SINCE anthrax is a disease exceedingly fatal to man and beast, and an important factor in several industries, further and more complete records of the circumstances of the outbreaks, and a better knowledge concerning the history and antecedents of the material conveying the infection, and of the natural condition and resistance of anthrax spores, should greatly facilitate preventive measures.

Anthrax is not only formidable from its severity, and the frequency of a fatal issue, but also because of the insidious nature of the attack.

The bacillus of anthrax is comparatively easily destructible, but it has the power of producing highly resistant spores, which gives the clue to the power of raw animal products to transmit the infection ; and since the environment of grease, dirt and animal discharges increases the natural resistance of the spores, efficient disinfection is the more difficult.

Notification of cases of anthrax occurring in workshops and factories in Great Britain became compulsory in 1895<sup>1</sup>, from that date to 1907, a period of 13 years, 514 cases were notified.

Human anthrax as it occurs in this country may be divided into non-Industrial, and Industrial or notifiable.

The "non-Industrial cases" include (a) those which occur amongst the numerous class of persons who come into contact with animals infected with anthrax during life, as shepherds, farmers, veterinary surgeons, etc., or have taken part in their slaughter, and subsequent disposal, as knackers, butchers, etc.; (b) rarer cases of transmission of anthrax from person to person by accidental contact, or of infection either during post-mortem examinations of a patient or animal that has died of anthrax, or during bacteriological research, or of infection by flies that have fed on infected carcases; (c) lastly, a number of people who become infected through contact or association with persons working under one or other of the industrial conditions to be mentioned. Several of these cases have been from time to time notified under the mistaken impression that they were industrial.

"Industrial Anthrax" includes cases arising from the manipulation (1) of wool (sheep or lambs' wool, goats' wool or hair [mohair], camels' wool or hair, alpaca and other allied textile fibres); (2) animal hair and bristles (pigs' bristles, horsehair, and less commonly the hair of other animals); (3) hides and skins; and lastly (4) cases arising in various other industries as harness, furniture, cutlery, boot, manure, rag-sorting and horn trades, grain portage, etc.

This paper is confined to the problem of anthrax arising during the manipulation of horsehair and bristles.

#### *Historical.*

In the latter half of the 18th century anthrax in the human subject came to be associated with the industrial manipulation of hides and fleeces.

In 1769 Fournier of Dijon drew attention to the occurrence of the disease in men who were handling raw hair and wool. Lawrence in 1847 described anthrax as it occurred in a hair factory in England, and in 1878 Dr J. B. Russell drew attention to the danger of anthrax in hair manufacture. He described nine cases occurring in connection with a horsehair factory in Glasgow. Seven of the cases were fatal,

<sup>1</sup> Factory and Workshops Act 1895, Sect. 29.

and seven of the internal type. Infection arose from Russian mane hair, and in his report he cites numerous accounts dating from 1777 published in France, of workmen having been attacked with anthrax and boils in opening and sorting bales imported from Russia.

Since 1878 no case of anthrax was known to have occurred in Glasgow until 1899, when three were reported, and in 1900, two were reported. The particular factory in which the early cases had occurred escaped, having, since 1878, discarded Siberian hair altogether.

In 1879 Dr J. H. Bell of Bradford definitely established the association of wool sorters' disease with anthrax by injecting blood from a case of the former into a rabbit, a guinea-pig, and a mouse. All the animals died within 60 hours, and the blood of each showed the presence of anthrax bacilli.

Frisch in Germany in 1887 proved that a case of rag sorters' disease was due to the anthrax bacillus. The *Annual Reports of the Medical Officers of Health of London* show that between 1873 and 1896 148 cases of anthrax were recognised in the metropolis and its neighbourhood. Of these 18 occurred in the manipulation of horsehair and bristles.

#### *Statistics.*

Anthrax due to industrial conditions became notifiable in 1895. From 1896 to 1898 (three years) 64 cases were notified, and of these three only, all occurring in 1898, were due to horsehair. That these three cases were all that occurred is improbable, the statistics being very likely incomplete owing to ignorance of the necessity of notification. Since 1898 the number of cases due to horsehair has varied from seven in 1903 and in 1905 to 19 in 1899 and the year 1898 may therefore fairly be taken as a starting point for compiling statistics.

The total number of cases of anthrax due to horsehair and bristles from 1899–1907 (nine years) was 106, making an average of nearly 12 cases in each year. Of the 106 cases 25 proved fatal giving a mortality of 23·5 %. The highest percentage of mortality, 40 %, was in 1906 when there were 10 cases and four deaths, and the lowest, 11 %, in 1901 when there were nine cases and one death.

In Dr Legge's *Report to the Chief Inspector of Factories* for 1904 the following statistics for the six years 1899–1904 are given:

Anthrax due to the manipulation of:

- (1) Worsted and wool, 88 cases, 23 fatal, 26·1 % mortality.

- (2) Horsehair and bristles, 70 cases, 17 fatal, 24·3 mortality.
- (3) Hides and skins, 86 cases, 21 fatal, 24·4 mortality.

Anthrax arising in other industrial conditions :

17 cases, 6 fatal, 35·3 mortality.

Completing these figures for the period 1905–1907 (three years) we find :

- (1) Worsted and wool, 87 cases, 24 fatal, 27·5 % mortality.
- (2) Horsehair and bristles, 34 cases, 9 fatal, 26·5 mortality.
- (3) Hides and skins, 45 cases, 13 fatal, 28·8 mortality.

And in other industries, 20 cases, 7 fatal, 35·0 mortality.

These figures disclose several interesting points (1) the increase in the number of cases in the worsted and woollen industries in recent years, notwithstanding the introduction of special rules and regulations for the manipulations of raw animal products likely to be contaminated with anthrax spores. This increase of cases is probably due to the increased quantities of dangerous wools handled as there was a demand for a particular class of yarn which could only be supplied (without dyeing) by using certain Central Asian wools, and with the demand broke out the attacks of human anthrax in the trades which used them. Scotch houses used none of this wool and were free from the disease. There may also have been an increase of the disease among animals in Central Asia but statistics are untrustworthy. (2) The higher mortality, despite the use of better methods of treatment. (3) Cases arising in the manipulation of horsehair and bristles are slightly less fatal than in the other industries. (4) The high mortality in the group of unnamed industries; this may be explained by the very varied nature of the industries in which no doubt anthrax not being expected is not watched for, the result being the diagnosis is too late for successful treatment.

The total cases of industrial anthrax from 1899–1907 from all causes was 452. Of these 114 died giving a percentage mortality of 25·2. The proportion of cases in each industry was wool, 4; bristles and horsehair, 2; hides and skins, 3. Other industries 1. (Table I.)

Cases of "non-Industrial anthrax," not being notifiable, can only be traced when a fatal result ensues. Table II shows the total number of deaths, 58, due to anthrax during the three years 1902–1904, of these deaths 32 were due to industrial conditions, the difference 26, is therefore the number of deaths occurring in non-industrial occupations. The average mortality of anthrax being about 25 % we may therefore estimate that during the three years above mentioned the non-industrial

TABLE I.

Year	All cases of Industrial Anthrax	Deaths	Cases due to the manipula- tion of Horse- hair and Bristles		Deaths	Percentage fatal
			Percentage fatal	Cases due to the manipula- tion of Horse- hair and Bristles		
1899	55	14	25·4	19	4	21·0
1900	37	7	18·9	15	4	16·0
1901	39	10	25·6	9	1	11·0
1902	38	9	23·6	10	2	20·0
1903	47	12	25·5	7	1	14·3
1904	50	11	22·0	12	4	33·3
1905	51	18	30·5	7	1	14·3
1906	67	22	32·8	10	4	40·0
1907	58	11	19·0	17	4	23·5
Total	452	114	25·2	106	25	23·5

cases amounted to about 104; this gives an average of over 34 cases per annum, as against 50 cases per annum due to industrial conditions. Also "industrial anthrax" accounting for 32 out of 58 deaths gives a percentage of industrial deaths of 55, and seven of the 58 deaths were ascribed to the manipulation of horsehair and bristles, hence 12% of the total deaths from anthrax occurred in the latter industries (Table II.)

TABLE II.

*Deaths from Anthrax 1902–1904 (3 years).*

Year	(1) Returns of Registrar General												(2)				(3)				(4)			
	England and Wales			Scotland			Ireland			Total for United Kingdom			Fatal cases noti- fied under Sec. 29 Factories and Workshops Act, 1895				Fatal cases of non-industrial Anthrax — dif- ference between (2) and (1).d				Fatal cases due to Manipulation of Horsehair and Bristles			
	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
1902	12	1	13	0	1	1	1	0	1	13	2	15	8	1	9	5	1	6	1	1	2			
1903	17	1	18	0	0	0	0	1	1	17	2	19	11	1	12	6	1	7	1	0	1			
1904	18	2	20	3	0	3	1	0	1	22	2	24	9	2	11	13	0	13	2	2	4			
Totals	47	4	51	3	1	4	2	1	3	52	6	58	28	4	32	24	2	26	4	3	7			

M = Males.

F = Females.

An analysis of the sex and numbers of persons employed in relation to the cases of anthrax in horsehair and bristle manipulations is instructive showing :

- (1) that anthrax is more fatal to women than men ;
- (2) that the occupational risk is greater for men than for women, both in horsehair and bristle industries, the explanation being that men are employed more in the earlier and more dangerous manipulations ;
- (3) that the occupational risk is greater and the mortality among the workers higher in the manipulation of horsehair than in brush-making ;
- (4) that the risk of infection from horsehair is very much greater than from bristles.

Of the 106 cases of anthrax due to horsehair and bristles between 1899 and 1907, 73, of which 15 were fatal occurred in males, and 33, of which 10 were fatal occurred among females. There was therefore a higher mortality in the cases among females, *i.e.* 30·3% to 20·5% among males.

Of these 106 cases, 60 with 13 deaths occurred in adult males, 24 with eight deaths in adult females, 12 with one death in young male persons, eight with two deaths in young female persons, and the remaining two cases were children, one, age 11, who recovered, and the other, age eight, who died.

For every 100 males attacked about 45 females contract anthrax.

In 1900 there were employed in horsehair manufactures 1412 work-people; in 1901, 2206, and in 1906, 2535, taking the average, 1900–1906, the number was 2076. Of these about 690 were employed in spinning and the incidental processes, 1035 in weaving, and its incidental processes, and 350 in dressing, finishing and miscellaneous processes. In 1901 out of 2206 workers—724 were males and 1482 females.

The total number of cases of anthrax that occurred in horsehair factories or workshops during the years 1901–1906 (six years) was 37 (seven fatal) of these 26 (five fatal) were males and 11 (two fatal) were females.

From these figures we may deduce the risk to, and the mortality among, the work people manipulating horsehair thus :

Average No. of people employed 1900–1906 = 2080.

Average No. of cases per ann. of anthrax =  $\frac{37}{6} = 6\cdot16$ ,

then the occupational risk expressed in percentages would be

$$\frac{6.16 \times 100}{2080} = .296\%.$$

In a similar manner we may estimate the risk for male workers only, *i.e.* .593% and for female workers .123%.

Also the mortality per 100 workers:

Male and female .168.

Male .115.

Female .022.

In other words for every 1000 workers, male and female, there are on the average nearly three cases of anthrax each year, and for every 1000 male workers six cases, and for a similar number of female workers just over one case. Hence the risk for men is more than five times that for women.

It is impossible to estimate accurately the number of people employed on bristles. The brushmaking industry includes practically all bristle workers, as well as a small unknown number of horsehair workers. Some of the cases of anthrax under the head of brushmaking are due to horsehair, making the occupational risk for brushmakers higher than it would be for bristle workers only; but as many brushmakers work on materials that have no connection with anthrax, it is possible to make a rough comparison as to the risks in manipulation of horsehair and bristles. In 1900 there were 5365 people employed in brushmaking—in 1901—7026 and in 1906 the number had risen to 11,753. Therefore the average number employed from 1900—1906 was 8048. In 1901 out of 7026 workers 4138 were males and 2888 were females.

The total number of cases of anthrax in brushmaking factories and workshops during 1900—1906 was 14 (four fatal). Of these 10 (three fatal) were in males and four (one fatal) in females; hence the occupational risk to brushworkers expressed in percentages was .029 and mortality per 100 workers .008.

Risk to male workers only .04% mortality .012%.

Risk to female workers .022% mortality .005%.

Therefore for every 10,000 workers of bristles we have nearly three cases of anthrax compared with the same number of cases in 1000 workers of horsehair, hence roughly the chances of infection from horsehair are 10 times as great as for bristles.

For every 10,000 male workers in bristles we have four cases of anthrax and among the same number of female workers just over two,

the risk for men then is almost double that for women as compared with horsehair in which the risk for men is five times as great as for women.

Dr Legge put the occupational risk during the five years 1899-1903 at 1·5% or 3% per annum in horsehair industries, and compared it with the woollen industry; estimating the risk (as in the case of horsehair) on the total number of persons employed. The risk worked out at 0·0028% or 0·00056% per annum. In horsehair industries practically all the employees are exposed to risk of infection; in the case of the woollen industries, as Dr Legge points out, the number employed is above half-a-million, males to females being roughly as two to three. Of this half million only 1171 (1164 males, seven females) are classed as being engaged in sorting, and 3093 (1882 males and 1211 females) in combing dangerous wools; making in all 4264 persons incurring special risk.

Estimated on this number of workers Dr Legge states that the occupational risk is 1·3% for five years, i.e. 26% per annum.

This figure is below the occupational risk in horsehair as worked out by Dr Legge, i.e. 3 and myself, i.e. 296.

The premises in which 77 of the 106 cases due to the manipulation of horsehair and bristles occurred were as follows:

Central horsehair warehouses	3	}	...	...	...	54
Horsehair factories	... 39					
<sup>1</sup> Hair dressing workshops	12					
<sup>1</sup> Brushmaking factories and workshops	...					15
Other industries as upholstering, mattress making, stuffing boxing gloves, and making knife cleaning machines	...					4
Infection conveyed to outside persons by workers	...					4
						<u>77</u>

These figures again indicate the much greater risk in the manipulation of horsehair, more especially, as previously shown, the horsehair industry is a much smaller one than brushmaking.

Of the 106 cases of anthrax due to the manipulation of horsehair and bristles only one was internal. There are no details of this case which occurred in 1900 and was fatal.

One fatal case in 1904 was of the erysipeloid type, there being no true pustule but a cellulitis of the neck. In one fatal case in 1906 intestinal anthrax developed secondarily to a pustule on the thigh.

<sup>1</sup> Six of the cases under these two heads occurred in domestic workshops or among homeworkers.

Out of 215 cases of anthrax arising from all industrial causes during four years, 1903-1906, 12, and one doubtful case, were of the pulmonary type, all in the woollen industry, and all were fatal except one in 1906. There was one fatal primary intestinal case probably from ingestion of spores with food. This patient was employed in some paint works. A very doubtful and non-fatal case of intestinal anthrax occurred in hide and skin industries in 1906. A fatal case of anthrax septicaemia (Anthracaemia) occurred in the woollen industry in 1905, there being no external lesion, and four cases of the erysipeloid form, of which only one was fatal.

These figures prove that the nature of the lesion varies considerably with the industry ; inhalation of the spores being uncommon in any but the woollen industry. This is a little remarkable, as many of the processes in the horsehair and woollen trades are very similar. There is however undoubtedly a great reluctance among men whose trade requires them to handle dangerous materials to state the true facts. A horsehair manufacturer, who has had considerable experience of anthrax, mentions two cases, both fatal, which were highly suspicious of pulmonary anthrax, and states that he is much more afraid of the internal than the external form. Pulmonary anthrax due to the manipulation of hides and skins one would *not* expect and does not find.

Table III shows the exact position of the pustule in 100 cases of anthrax due to the manipulation of horsehair and bristles from 1899-1907. From this it will at once be seen, that the pustule occurs most frequently on the neck, 34·2 %, then on the cheek, 20 %, then the forearm, 11 %, none of the other positions at all approach these in frequency.

It will be noticed too, that the vast majority of the cases (93 out of 100) occur on the exposed parts, and that of the exposed parts the neck and cheek (57 out of 93) are the most frequent sites. This may be explained in two ways : (1) the forearms and hands though more in contact with raw material are more frequently washed ; (2) the nails harbour dirt containing spores, the worker scratching his face or neck is then very liable to infect these parts. This is well illustrated by a case in which a worker on horsehair went home and accidentally scratched his niece's neck, with the result that she developed an anthrax pustule.

For the purposes of comparison, Table IV has been drawn up, showing that to some extent the situation of the pustule varies with the occupation.

TABLE III.

*Situation of the Lesion in 105 cases of malignant pustule due to manipulation of Bristles and Horsehair.*

Situation of pustule	Cases	Deaths	Percentage fatal	Position percentage
Head	...	3	0	2·8
Forehead	...	7	1	14·3
Eyebrow	...	2	1	50·0
Eyelids	...	2	0	1·9
Face	...	5	1	20·0
Chin	...	5	3	60·0
Cheek	...	21	2	9·5
Neck	...	36 <sup>1</sup>	12	33·3
Arm and shoulder	...	4	1	25·0
Forearm	...	12	1	8·3
Hand	...	0	0	0
Fingers	...	0	0	0
Trunk	...	2	0	1·9
Lower extremity	...	1	1	100·0
Not stated	...	5	1	20·0
Totals	...	105	24	22·8

It will be noticed that pustules on the head, face and neck, are the most common in the English cases, while in the foreign cases, lesions on the upper extremity are next in frequency to the head and face, and lesions on the neck come third. In all the lesions on the trunk and legs (covered parts) are uncommon. The statistics of Debray and Cavaillé refer to industrial cases while many of Koch's are agricultural (connected with disposal of carcases).

The statistics for wool and horsehair are much alike, but in hides the pustule was situated on the neck most frequently (60%), possibly due to the frequent carrying of hides on the shoulder.

In Koch's cases the majority of the pustules, under the title of upper extremity, were on the hands and fingers, while in malignant pustule arising in all industries in England 1903–1906, only three were on the hands and one on the finger.

In horsehair and bristle industries in England 1899–1907 there were none on hands or fingers.

The mortality of cases of malignant pustule varies with the situation; thus in the English and French statistics the neck would appear to be a much more fatal situation than head and face or other parts (Table V),

<sup>1</sup> Including one erysipeloid form without true pustule (fatal).

TABLE IV.

Position of the Pustule in different Industries and Countries.

\* Fingers and hands chiefly.

while in the Italian observations the neck only comes third, lesions of the head and face and trunk being respectively more common.

Dr Legge states that out of 84 pustules on the neck, 26 were fatal, 30·9 %, of 12 cases on the upper eyelid five were fatal 41·6 %, of 31 on the cheek three were fatal 22·5 %, of 14 on the forehead two were fatal 14·3 %, and of 23 on the wrist and forearm only one was fatal 4·3 %. Difficulty of operation, frequent absence of diagnostic signs of local necrosis, looseness of the cellular tissues may help to account for the high mortality when the upper eyelid or eyebrow is affected.

TABLE V.

*Mortality in relation to the situation of the pustule.*

Situation	United Kingdom Industrial Anthrax all causes 1903-1906 (4 yrs.)	United Kingdom Industrial Anthrax Horsehair & Bristles 1899-1907 (9 yrs.)	France Statistique de Cavallé (1902-1905)	? Italy Statistics of Nassarow quoted by Sclavo	
Head and Face	82 cases 17·07 % 14 deaths fatal	45 cases 17·7 % 8 deaths fatal	8 cases 37·5 % 3 deaths fatal		26·3 % fatal
Neck	79 cases 32·91 % 26 deaths fatal	36 cases 33·3 % 12 deaths fatal	3 cases 100 % 3 deaths fatal		18·5 % fatal
Trunk	1 case 0 % 0 deaths fatal	2 cases 0 % 0 deaths fatal	0 cases 0 % 0 deaths fatal		22·7 % fatal
Upper Extremity	28 cases 17·85 % 5 deaths fatal	16 cases 12·5 % 2 deaths fatal	8 cases 0 % 0 deaths fatal		13·8 % fatal
Lower Extremity	4 cases 25 % 1 death fatal	1 case 100 % 1 death fatal	1 case 0 % 0 deaths fatal		5·1 % fatal
Total	194 cases 23·6 % 46 deaths fatal	100 cases 23 % 23 deaths fatal	20 cases 30 % 6 deaths fatal	Out of 180 observations	

With the exception of Great Britain in probably all other countries in the world, anthrax in the human subject is derived more from contact with animals suffering from anthrax than from spores contained in wool, horsehair, hides and skins, and other raw animal products. Italy and Great Britain are the only countries whose governments require notification of anthrax cases in the human subject; and Great Britain, as has been shown, at present only requires industrial cases to be notified, while Italy makes no distinction between industrial and non-industrial anthrax, as far as notification is concerned; so that it is difficult to obtain reliable figures to compare with the English figures just given. Yet it is important to examine the prevalence of anthrax abroad, since all bristles and much of the horsehair used in this country are imported.

In Italy Prof. Monti says that anthrax in man is relatively more frequent than in any other European country. From 1890–1904 (15 years) there were no less than 36,426 cases with 7308 deaths. A percentage mortality of 20·6. The annual number of cases has increased but the death rate has decreased (Table VI).

TABLE VI.

*Anthrax in the Human subject, Italian statistics.*

Year	Cases of Anthrax notified in all Italy	Deaths from Anthrax in all Italy	Percentage fatal
1890	2047	526	25·6
1891	2241	645	24·31
1892	2077	650	31·3
1893	2461	598	24·3
1894	2400	635	26·46
1895	2179	621	28·5
1896	1985	453	22·82
1897	2123	460	21·66
1898	2327	433	18·6
1899	2672	461	17·25
1900	1867	330	17·67
1901	1992	341	17·11
1902	3528	403	11·42
1903	3423	397	11·6
1904	3104	355	11·43
Totals	36426	7308	20·6

Anthrax in Italy is generally of agricultural origin, there being in many districts, especially in the south, which is more agricultural, a close relation between the number of cases of animal and human anthrax. Sardinia, Calabria, and the Campania show the highest anthrax figures both animal and human.

The north of Italy is more industrial. Dr Corradi collected statistics, from 1877–1890, of 153 cases of malignant pustule in man, occurring in Genoa, one of the chief ports. Of these 117 were of industrial origin, and six of the 117 cases were among people employed on hair only, while 12 of the cases were in trades connected with skins and hair.

Bormans found, in studying an epidemic of anthrax in Turin, that the largest number of cases of malignant pustule occurred among tanners; next came workers employed in making hair pencils, painting and varnishing brushes, and lastly among the peasants who contracted the disease indirectly from the tanneries.

In France only 32 causes of death are entered ; anthrax is not one of these, hence no official statistics are available.

Information communicated by Messrs Déséglise & Co., of Paris, and by Messrs Carlo Pacchetti & Co., of Milan, who also do business in Paris, shows that deaths from anthrax due to the manipulation of horsehair and bristles are exceedingly rare in France, but that a few cases have occurred especially from handling Russian horsehair.

Chauveau in 1893 gave particulars relating to seven cases of anthrax. These were due to the manipulation of horsehair, probably Chinese. The cases were spread over a period of four months, two were of the gastro-intestinal type and two erysipeloid (cutaneous anthrax). Six of the seven cases proved fatal.

TABLE VII.

Adapted from a similar Table by Prof. Langlois of Paris, and from details published by Dr Debray (*Le Charbon Industriel*, 1906).

Statistics	Numbers employed	Occupational risk expressed in percentages per annum	Mortality per 100 workers per annum	Percentages of cases fatal
English Wool sorters, 1899—1903	4264	.26	.05	27.7
English Horsehair Workers, 1901—1906	2080	.296	.056	18.9
Le Roy des Barres, } Hair dressers 1875—1896 } St Denis } Leather dressers	160	.42	.09	20
Debray, 1897—1905, Ditto	760	.34	.04	12.3
Cavaillé, 1902—1905, Wool sorters and Teasers	1200	.5	.02	4.6
... ...	2168	.23	.07	30
St Jumien, Leather dressers	350	.4	.1	30

In Table VII French and English figures of industrial anthrax are compared. These figures are worked out from a series of 56 cases among English wool sorters, 37 in English horsehair industries, 15 in French horsehair industries and 57 among French leather dressers (Le Roy des Barres), 64 in French horsehair and leather industries (Debray), 20 among French wool sorters, etc. (Cavaillé).

These figures emphasize the greater risk both in England and France from horsehair (Debray's figures including horsehair) and further with the exception of Cavaillé's figures, only judged on 20 cases and none of these due to horsehair, the risk in the English industries of wool and horsehair is less than that in France, while the English mortality per 100 workers compares very favourably with the French mortality. The low percentage mortality in Debray's cases, 4.6, is remarkable and throws

some doubt on the genuineness of his cases of anthrax, more especially as he quotes 64 cases in nine years (1897-1905) to Le Roy des Barres' 72 cases in 22 years (1875-1896) in the same district (St Denis).

In Germany the horsehair and bristle industries are probably larger than in England. In 1895 Kübler estimated that 12,000 people were employed in the German empire in horsehair, brush and broom factories. Compare with this number the 6777 engaged in similar industries in England in 1900 (horsehair 1412 and brush-making industries 5365) and with 13,288 in 1906 (2535 horsehair and 11,753 brush industries), and there is not the least doubt that the German industries mentioned have increased as fast if not faster than the English ones.

In a paper published in 1886, W. Koch mentions 1473 cases of malignant pustule with 472 deaths, a percentage mortality of 32. From the Annual Report on diseases of animals in Germany we find that from 1894-1903 (10 years) there were 901 cases of human anthrax from all causes (anthrax not being notifiable throughout Germany the figures must necessarily be too low), *i.e.* just over 90 each year compared with my estimate of about 84 cases per annum in Great Britain. Of these 901 cases, 128 were fatal, a percentage mortality of 13·9, 26 of the 901 were ascribed to the manipulation of bristles and horsehair, while only 11 of the others were due to industrial conditions, that is only 4·1% in Germany from 1894-1903 were industrial as compared with over 50% in England (1902-1904) and 2·8% were due to horsehair as compared with 11·5% in England.

Kübler says that from 1880-1896 there were more than 141 cases of anthrax due to bristles and horsehair, of which 44 were fatal, mortality 31·2%, and that these figures are too low because anthrax is not notifiable all over the Empire and because of errors of diagnosis. He strongly holds the opinion that bristles as well as horsehair are a source of infection and quotes cases to prove this assertion.

Monti in a paper on the infections of work people, states that in Prussia from official data from 1898-1902 (five years) there were 378 cases of anthrax with 44 deaths, a percentage mortality of 10·6, but gives no details as to the nature of employment of the cases.

Sclavo in a paper published in 1903 mentions 91 cases of anthrax due to horsehair in Germany of which 29 were fatal, per cent. fatal 31·6.

Debray gives the following for Germany:

			Cases	Deaths	Per cent. fatal
Spinning of Horsehair	...	...	11	6	54·5
Brushmaking	...	...	2	2	100
Tanneries	...	...	25	4	16
Manufacture of hides and skins	...	...	11	0	0
Wool combing	...	...	1	0	0
Knackers	...	...	2	0	0
			52	12	23·0

This excludes cases in the Palatinate where the mortality is above 23%.

TABLE VIII.

Nature of trade, Country, Dates	Numbers em- ployed	Cases of Anthrax	Occupational risk expressed in percentages per annum	Mortality per 100 workers per annum	Percentages of fatal cases
English Horsehair Workers, 1901—1906	2080	37	·296	·056	18·9
German Empire Horsehair Factory at Schleswig, 1873—1881 ...	35	25	8·7	3·9	44
German Empire. Three Horsehair Fac- tories in Eschwege, 1884—1888 ...	30	16	10·6	4·0	37·5
German Empire. Three Horsehair Fac- tories at Kitzingen, 1890—1894 ...	150	19	2·5	·8	31·6
English Brush manufacturers, 1901— 1906 ...	8048	14	·029	·008	28·5
German Empire. Brush makers in Nuremberg, 1890—1894 ...	1700	19	·22	·034	15·8

Table VIII shows the occupational risk and mortality per 100 workers in certain horsehair and brush manufacturing towns in Germany and the figures are tabulated with similar English figures for comparison.

The higher occupational risk and mortality per workers in Germany is remarkable, though allowance must be made for the smaller numbers employed in the particular instances given.

Moreover, the mortality of cases varies considerably from 10·6 of Monti to the 32% of Koch, yet the average would not be far from the English mortality. The mortality in horsehair seems somewhat higher than the English figures.

Eppinger records many internal cases previous to 1886 with a mortality of 88%, and more recently in Vienna there were six cases (four fatal) among brushworkers due to Russian horsehair.

Munch and Albrecht have recorded cases of the gastro-intestinal form among horsehair workers in Russia.

Reports as to anthrax in China are very contradictory. At the

Catholic Hospital, Hankow (from which port several thousands of pounds' worth of bristles are annually exported) 15,000 natives are seen each year, but only three or four cases have occurred in the past seven years. (Report of Medical Officer to the Consulate, Hankow.)

In Tientsin, through which city passes large quantities of bristles, no cases of anthrax have been heard of. Several medical men in various parts report that they have not seen any anthrax cases, though in several of these places hides and bristles are prepared.

Boils and swellings are said to be common after sorting horsehair, but are never fatal. There is, however, no registration of births or deaths in China, and the Chinese, except Christians, do not go to the foreign doctors, which perhaps may partly account for the supposed absence of anthrax.

On the other hand a doctor, who had spent several years in China, reports that he was seldom without a case. Anthrax was constant, but in all probability the well-known symptoms lead to the men attacked immediately recognising the disease and seeking surgical assistance without delay.

In the hospitals of Montevideo, South America, about 20 cases of anthrax are treated annually, and in the central produce market of the Argentine it is stated that no case of human anthrax has been known, though millions of hides and skins, and about 150,000 tons of wool, pass through it. This statement must be accepted with caution, since dry hides from South America have certainly given rise to cases of anthrax in Antwerp and Liverpool.

In Buenos Ayres "grano malo," a pustular swelling like anthrax, is said to be well known.

Variations in the prevalence and mortality of anthrax among human beings may possibly to some extent be accounted for by natural constitution, inhabitants of colder climates being probably more resistant than those of warmer, such as Italy.

The more sluggish temperament of the Englishman may tend to reduce the number of cases, but renders him, as will be shown later, less susceptible to the serum treatment than the Italian, *i.e.*, it takes a more virulent form of the disease to affect an Englishman than an Italian and therefore it takes more serum and earlier injection to obtain a favourable termination.

Also in the case of the Chinese it is possible that, their diet being largely vegetarian, a condition of body is created which makes the disease less fatal, but it is certain it largely exists.

*History of bristles and horsehair in relation to anthrax.*

The danger of infection from anthrax in the manipulations of animal hair and bristles depends on the origin, kind, and cleanliness of the materials, and the processes they have to undergo.

*Bristles.* Bristles are obtained entirely from the hog, and those worked in this country are all foreign. The English pig is useless as a producer of bristles, because by intermixing and crossing, swine have lost in the value of their clothing what they have gained in the delicacy of their flesh.

In the more northerly parts of Europe and Asia the hogs have longer and thicker coats. The larger and older the hog, and the more nearly he approaches to the wild boar, the better his bristles. A long, spare and thin pig will produce long and stiff bristles; the best are taken from along the dorsal spine, each pig yielding about  $\frac{1}{2}$  lb. of bristles. Bristles are taken from both live and dead animals. Most bristles are imported into Great Britain partially prepared, that is, roughly combed by drawing through graduated steel combs fixed upright on a bench and then bundled according to length, quality and colour. A small quantity comes in bleached or dyed and well cleaned, bundled and packed. Up to within the last 25 years Russia, Siberia and Poland held the monopoly of supply; but with the opening up of new ports the bristle trade in China has increased enormously, until at the present time Great Britain imports more bristles from China than from any other country. Germany also largely supplies the English brushmaking industries, and smaller quantities come from Hungary and Austria, the Balkans, France, India, Japan, and North and South America.

Through the vast forests of Northern Europe, where trees bearing berries, acorns and other fruit drop endless supplies of food, wander large droves of swine, attended only by a rude swineherd. In Russia at certain seasons of the year those animals likely to produce the best bristles are fattened on tallow waste for a short time and then plucked alive. The swine protected from the rigours of the climate by the short thick tufts, known as pig's wool, which grow at the root of the bristles, wander northward again until a fresh crop of hair has grown. As the swine roam through the forests they rub themselves against trees at the foot of which fall quantities of hair. These are collected by the swineherds, sewn up into skins, and sold to collectors who carry

them to the bristle markets; from these they pass to the two great distributing centres, Moscow and Leipzig.

From the dead pig the bristles are scraped off after scalding, or dragged off after softening by wood ashes.

Bristles come from all parts of Russia and Siberia, but Polish bristles, whether from natural deterioration or careless dressing, are now of small value. Bristles from France, Germany, and Southern Europe vary greatly, some German States supplying hair of good length and quality, but others with France and Southern Europe yield either too long a bristle, or else too short and rigid a one.

From France too is imported a very highly esteemed white bleached bristle, but this is probably largely manufactured Russian. American bristles are wanting in flexibility and are not used now to any great extent.

Bristles from China and India have only been in use about 25 years, and the more valuable bristles come from Northern China. There the pig, having been "stuck," is scalded and the bristles scraped off; these are washed in hot water, air dried, roughly bundled in the interior, and sold to bristle merchants or dressers at Tientsin, Hankow, etc., who open the bundles, comb and straighten the bristles, sometimes by steam. They are then resorted and bundled according to length and quality, care being taken to get the "bend" (the natural curve of the bristle) all the same way in each bundle. Some dressers use a dry powder (naphthalene) in packing. The better class bristles are packed in rice paper stamped with the dresser's name or mark.

The waste that falls to the ground is roughly bundled and sold as riflings; it is exceedingly dirty, but as bristles are sold on gross weight, the more dirt the better, so far as the Chinaman is concerned. Much of the dirt in bristles is doubtless gathered in the drying in the open, and in dressing before exportation bristles pass on an average through 10 pairs of hands, and the washing and combing largely remove the original animal fat and dirt.

Bristles from Japan, like the best Chinese, are well cleansed and packed.

The cleanliness of the bristles when they reach the manufacturer varies widely, some being extremely dirty, as Russian, Siberian, and the Chinese riflings; the dirt consists of dust of all kinds, including the irritating naphthalene, animal dirt, skin and scurf, nits, etc., others, as the better class Chinese, French and Japanese, are very clean. Bristles differ immensely in substance, colour and length, varying from jet black

*Industrial Anthrax*

TABLE IX.

Name by which britties are known in the Trade	Country of origin	Qualities	Length	Colours	Condition when imported	How imported	Remarks
Russian	Russia	1. Firsts 2. Suchoy's 3. Seconds or Donskois	3" rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " sizes to 5 $\frac{1}{2}$ "	White, yellow, black & grey	Combed and bundled to sizes & qualities	In cases	Very dirty, full of dust.
Siberian	Siberia	1. O'Katka 2. Firsts 3. Suchoy's 4. Seconds	3 $\frac{1}{2}$ " rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " sizes to 4 $\frac{1}{2}$ " 5 $\frac{1}{2}$ ", 6", 6 $\frac{1}{2}$ ", 7" 3 $\frac{1}{2}$ ", 4", 4 $\frac{1}{2}$ ", 5 $\frac{1}{2}$ 3", 3 $\frac{1}{2}$ ", 4"	"	"	"	"
Polish	Poland	Aftall	3 $\frac{1}{2}$ "	Yellow, white and grey	"	"	Dirty and dusty.
Auszug	Bohemia	Spitz-Spitz One only known as Auszug	4", 4 $\frac{1}{2}$ " 4 $\frac{3}{4}$ " rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " sizes to 7" 5" rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " to 7"	"	"	"	Rather dirty and dusty.
German-Polish	? Austria	Leck	4 $\frac{1}{2}$ "	White, yellow, black & grey	"	"	"
Indian	The Balkans India	Indians Border-Indians	Bukharest Calcutta 2" rising by $\frac{1}{4}$ " sizes to 8"	"	"	"	"
German	Germany	All qualities known as German unprepared	3 $\frac{1}{2}$ " rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " sizes to 7"	"	"	"	"
French	France and possibly Russia	German prepared and bleached	3" rising by $\frac{1}{4}$ " & $\frac{1}{2}$ " sizes to 7" Known by colour 50 mm. to 120 min.	White	Bundles bleached and fully prepared	"	Well prepared, cleaned and packed.
Chinese	China	as Beau, Blane, Demi-blanc, Gris, "Lily White, the best	Hankow 2" rising by $\frac{1}{4}$ " up to 7". Ruffles (waste) 2" or less	Black	Except ridings very well bundled and prepared	"	Ridings very dirty, others very clean.
Japanese	Japan	Tientsin Hong-Kong	Ditto A. Long B. Medium C. Short	"	"	"	"
American	North and South America	?	?	?	Bundled and fully prepared	?	Very clean not much used.

through dark or light grey to yellowish buff or white. The length varies from two to 14 inches. The bundles weigh from a few ounces to two or three lbs., and vary according to length, quality and colour.

Table IX is a classification of bristles as imported into England.

*Horsehair.* Horsehair is derived from the manes and tails of English, Russian, Siberian, Australian, South American and Chinese horses. In all countries it is taken from both live and dead animals, and a bale may contain both "live and dead" hair.

English horsehair is largely derived from combings from living horses. In China 50% of the hair exported comes from Koolan in Mongolia, where the hair is taken mainly from dead horses; most of the other 50% is "live hair" and comes from around Mukden in Manchuria. In Russia, Siberia and China many of the horses are wild, but a few are farmed for the hair. Whatever its origin, the hair may be either directly made into bales of various sizes for export, or may undergo some preliminary processes before export. In this case the hair is washed in cold water, and manes are roughly separated from tails. The long tail hair is more valuable, and therefore is often well washed and beaten, and sometimes dyed. Chinese horsehair is roughly combed, the waste, corresponding to bristle riflings, is exported as "combings." Hair is sold by gross weight and therefore the measures to get rid of the dirt are not so energetic as they ought to be. From South America the majority comes from the Argentine Republic and Uruguay and is often mixed with cow tail hair. Smaller quantities are occasionally imported from Hungary, Japan and Morocco.

Russian and Siberian tail hair arrives in loosely packed bales of about 4 cwt. each, roughly bundled and all colours packed together. Mane hair in loosely packed bales of 2½—5 cwt. each, all colours together, but a little cow hair is mixed in.

Chinese and Manchurian tail hair comes in cases of about 1½ cwt. The hair is wholly or partially drawn into lengths, and colours separated. Mane hair arrives in cases of about 1½ cwt. treated in the same way as the tail hair, combings in bales of 5 cwt.

South American tail hair arrives in press packed bales of 8—9 cwt. sometimes wholly on the dock but more often free. Mane and tail hair also arrives, all qualities and colours mixed. Cowhair is often mixed with the horsehair.

*Mode of importation.* All bristles and horsehair are imported into England by brokers, arriving in casks, cases or bales. Practically all of it enters at the Port of London, and for a time is centred in two ware-

houses close to one another. Here certain cases are opened for the purpose of collecting samples, and the consignments are sold by auctions, which are generally held once a fortnight. The raw materials then pass directly to the manufacturers. None of the cases are ear-marked by any sanitary authority as are consignments of tinned goods, so that there is no restriction on the distribution of any infected material.

From this brief survey of the antecedents of bristles and horsehair it is evident (1) that bristles enter Great Britain in a far more prepared state than horsehair, (2) that tail hair is better cleansed than mane hair.

#### *Manipulation of bristles and horsehair.*

Bristles are mainly used for making brushes, a very small quantity is used in the same way as short horsehair. Long horsehair above 18 inches is used for weaving, seating, and for making crinolines, below 18 inches chiefly for brushmaking. The short tail hair is used for upholstery and mattress work, and the short mane hair for horsehair carpets, oil press cloth, etc.

The processes to which hair is subjected vary with the nature and origin of the hair. Russian tail hair is first sorted for colours, then wet hackled, *i.e.* drawn while damp by hand through upright steel combs, afterwards drawn into lengths and bundled. Russian mane hair is sorted for colours, then carded (a carding machine consists of rollers with metal teeth revolving against each other) for spinning or curling. Chinese tail hair if not completely drawn, must have the process completed; the mane hair if in cases must have strings removed from bundles before carding.

South American tail hair is cut off docks when necessary, wet hackled and drawn into lengths. The mixed hair is first sorted for colour, quality and length, and the long and short hair is then treated in the same way as the Russian.

For curling, the raw materials are taken in the required proportions and placed in a willeying machine which consists of a cylinder with several teeth on it; in this the material is further cleansed, the dust extracted, and thoroughly mixed; the machine is usually covered and connected with a fan. The mixed material is then spun, and the curl set by steam or by baking in a fire-heated oven.

*Brushmaking.* Most brushmakers buy horsehair already prepared from the horsehair manufacturer; a few of the larger firms prepare their own, selling all that is of no use for brushes for other purposes; some

bristles are used as imported, and made directly into brushes without any further processes, but for other brushes it is necessary to blend and dress the bristles to the required length and stiffness. Sorting for stiffness is accomplished by drawing the bristles through a series of graduated steel combs. In sorting into lengths a handful of mixed bristles is stood up against an inch measure, marked to the eighth parts of an inch; then the bundles are held in the left hand, and with the right finger and thumb bristles above the indicated length are removed. This is repeated until the residue is as solid as requisite. Each bundle is now so accurately arranged that the flag ends of the bundle are almost solid and perfectly level, while the base of the bundle is smooth. It is quite common during the above operation for the sorter before pulling the bristles to wet his finger and thumb from his lips, a most dangerous practice as the following case illustrates. In 1900, a girl died from anthrax; she had a pustule on the cheek, and large pustules were found in the stomach and intestines, but nowhere else, hence the lesion in the latter positions was probably due to a separate infection. This girl was in the habit of constantly chewing hair.

Table X. outlines the processes of brushmaking. Washing and bleaching are done in some cases before and in others after the brush is made; in either case the process is the same, though it varies in different factories. In the washing, soap and boiling water or washes made of oleic acid 1 in 8 and .880 ammonia 1 in 20 at 190° F. are used, and the bristles or brushes rubbed by hand on corrugated beech blocks or stone. Bleaching is done by sulphur. Many of the processes both in horsehair and brush-manufacturing are extremely dusty, and it is remarkable there have been so few cases of internal anthrax recorded.

#### *Dangerous processes and materials.*

Table XI shows the occupation of cases of anthrax ascribed to infection from horsehair and bristles from 1899-1907 (nine years).

From this will be seen that cases have occurred in practically all the processes to which hair is subjected; in the warehouses where the hair is stored after arrival, in the course of distribution, and in most of the subsequent processes even from finished products. Further, it will be seen that the incidence is greater (1) among workers in short hair, as brushmakers; and (2) in the earlier processes, as drawing.

Lastly, seven of the cases, of which two were fatal, were due to infection carried to outside persons by workers in horsehair. Kübler quotes

TABLE X.  
*Table showing materials and processes in brushmaking*

Raw materials	Preparation of raw material	Actual brushmaking	The finished brush
<b>Handles and backs of brushes consist of</b> Various woods, Silver, pearl, Tortoise-shell, Ivory, bone, quill, and Composition.	Cutting, Weathering, Planing, Shaping, Boring, and Drilling.	Drawn work, filling by hand and trepanning using wire, making scrubbing, nail, cloth, tooth, hair and other brushes.	Household, trade, and ma- chinery brushes.
<b>Brush consists of</b> Hair of Horse, Cow, Bear, Squirrel, Koinsky, Badger, etc.	May be rough dressed (abroad), Dressing, Sorting, Drawing, Mixing, Washing, Bleaching.	Machine work, filling by machinery nearly all varie- ties of brushes.	Fancy brushes, as toilet, hair, nail and shaving brushes.
<b>Accessories</b> Steel and other wire, Pitch, Wax, etc.	Set work—Pan department, filling brooms by hand, using string and pitch.	Trimming Washing Bleaching Finishing Polishing	Bone brushes—tooth and nail. Brushes for painters and white- washers. Artists' Brushes.
			—

TABLE XI.

*Showing occupation of cases (Horsehair and Bristles).*

Occupation	c=cases, d=deaths.										Total c—d
	1899 c—d	1900 c—d	1901 c—d	1902 c—d	1903 c—d	1904 c—d	1905 c—d	1906 c—d	1907 c—d	1908 c—d	
Making boxing gloves	—	1—0	—	—	—	—	—	—	—	—	1—0
Brushmaking	—	3—2	2—1	2—1	3—0	2—0	—	1—1	—	—	13—5
Carding	4—1	3—1	—	—	—	—	—	—	—	—	7—2
Carrying	1—0	—	—	—	—	1—0	—	—	2—0	—	4—0
Clerk	—	—	—	1—0	—	—	—	—	—	—	1—0
Curling	5—1	—	—	—	—	1—0	—	2—0	—	—	8—1
Drawing	2—1	—	1—0	1—0	—	1—1	2—1	2—1	2—0	—	11—4
Dressing	—	1—0	—	—	1—0	1—0	1—0	—	1—0	—	5—0
Fibre drawing&dressing	—	1—0	—	—	—	—	—	1—0	—	—	2—0
Hackling (3 of the cases were wet hackling)	—	—	—	—	—	1—0	1—0	—	2—0	—	4—0
Making hair cloth	—	—	—	—	—	—	—	1—0	—	—	1—0
Labourer	1—0	1—1	—	2—0	—	1—0	—	—	—	—	5—1
Making mattresses	—	—	—	1—0	—	—	—	—	—	—	1—0
Mixing	1—0	—	—	—	—	—	—	—	—	—	1—0
Not stated	—	—	1—0	—	—	—	—	3—2	1—1	—	5—3
Plasterer	—	—	—	—	—	—	—	—	1—0	—	1—0
Sorting	2—1	—	—	—	—	—	1—0	—	2—2	—	5—3
Spinning	—	—	—	—	—	—	—	—	1—0	—	1—0
Teasing	—	—	1—0	—	—	—	—	—	—	—	1—0
Twisting	—	—	1—0	—	—	—	—	—	1—1	—	2—1
Vanman	—	1—0	—	—	—	—	—	—	1—0	—	2—0
Warehouseman	—	1—0	1—0	1—0	1—1	—	—	—	—	—	4—1
Washer	—	—	—	—	—	—	1—0	—	1—0	—	2—0
Weigher	1—0	1—0	—	—	1—0	—	—	—	—	—	3—0
Willeyer	—	—	1—0	—	—	—	—	—	—	—	1—0
Willowing	—	—	—	1—0	—	1—1	—	—	—	—	2—1
Yarn winding	—	1—0	—	—	—	—	—	—	—	—	1—0
Outside cases	2—0	—	1—0	—	1—0	2—2	—	—	1—0	—	7—2
Drawing & sorting	—	1—0	—	—	—	—	—	—	—	—	1—0
Warehouseman & brushmaker	—	—	—	1—1	—	—	—	—	—	—	1—1
Hackler & brushmaker	—	—	—	—	—	1—0	—	—	—	—	1—0
Drawer & brushmaker	—	—	—	—	—	—	1—0	—	—	—	1—0
Drawer & wet hackler	—	—	—	—	—	—	—	—	1—0	—	1—0
Totals	19—4	15—4	9—1	10—2	7—1	12—4	7—1	10—4	17—4	106—25	

many similar cases in Germany, as does Debrey among French workers.

The case in mattress making is an excellent example of the vitality of anthrax spores. A hair mattress was being renovated after twenty

TABLE XII.

*Nature of material giving rise to infection in 106 cases. (Horsehair and Bristles.)*

Material	1899 c—d	1900 c—d	1901 c—d	1902 c—d	1903 c—d	1904 c—d	1905 c—d	1906 c—d	1907 c—d	Totals c—d
Not stated ...	6—1	3—0	4—0	b2—0	—	1—0	1—0	2—0	3—0	22—1
Australian hair ...	—	1—0	—	—	—	—	—	—	—	1—0
Siberian hair ...	—	—	—	—	—	2—0	—	1—1	1—0	4—1
Chinese manes and short hair ...	7—2	—	2—1	4—2	1—0	—	—	—	1—1	15—6
English cow hair ...	1—0	—	—	—	—	—	—	—	1—1	2—1
Chinese hair ...	1—1	2—0	—	—	—	1—0	2—1	1—1	1—0	8—3
American hog hair ...	—	—	—	—	—	—	—	—	1—0	1—0
South American hair ...	2—0	—	—	—	—	—	—	—	—	2—0
English horsehair ...	—	1—0	—	—	—	—	—	—	1—0	2—0
Chinese, Russian & American hair ...	—	3—2	—	—	—	—	—	—	—	3—2
Russian tail hair ...	—	—	—	—	—	—	—	—	1—0	1—0
Russian bristles ...	—	—	—	—	—	—	—	—	1—0	1—0
Russian hair ...	—	—	—	—	—	—	—	—	1—0	3—1
Bristles ? Origin ...	—	1—1	—	—	—	—	—	—	—	1—1
British cow hair and horsehair.										
American hog hair ...	—	—	—	—	—	—	—	—	1—1	1—1
Chinese, Siberian and Russian hair	—	—	—	—	—	—	—	—	a1—0	1—0
Chinese and South American hair	—	1—0	—	—	—	—	—	—	—	1—0
American hog hair, English cow tails all dyed and boiled ...	—	—	—	—	—	—	—	—	1—0	1—0
Chinese horse tails ...	—	—	2—0	—	—	—	—	—	—	2—0
Chinese and Siberian bristles ...	—	—	—	—	—	—	—	1—1	—	1—1
Russian and South American hair	—	1—0	—	—	—	—	—	—	—	1—0
Chinese and Siberian hair ...	—	—	—	—	1—0	2—2	—	1—0	—	4—2
Siberian tails ...	2—0	1—1	—	—	—	—	—	—	—	3—1
Argentine and Russian hair ...	—	—	—	—	—	—	—	1—0	—	1—0
English, S.American and Russian hair	—	—	—	—	—	—	—	1—1	—	1—1
Russian and Siberian hair ...	—	—	—	—	1—0	1—1	—	1—0	—	3—1
Chinese hair and bristles ...	—	—	—	—	1—0	—	1—0	—	—	2—0
Yak hair, Chinese, Siberian, and Australian hair ...	—	—	—	—	—	—	3—0	—	—	3—0
Russian and Chinese hair or bristles	—	—	—	—	—	1—0	—	—	—	1—0
Russian and Chinese hair ...	—	1—0	—	—	—	2—1	—	—	—	3—1
South American horse and cow hair (tan yards) ...	—	—	—	—	—	1—0	—	—	—	1—0
Siberian and Chinese horsetails ...	—	—	—	—	—	1—0	—	—	—	1—0
Chinese horsehair, Indian and other bristles ...	—	—	—	—	—	1—1	—	—	—	1—1
Native ...	—	—	—	—	—	1—0	—	—	—	1—0
Chinese and Russian bristles ...	—	—	—	—	1—0	—	—	—	—	1—0
All hair except Chinese ...	—	—	—	4—0	—	—	—	—	—	4—0
S.American, Chinese & Siberian hair	—	—	1—0	—	—	—	—	—	—	1—0

N.B. Unless otherwise stated hair means horsehair.

a = The Chinese had been disinfected.

b = In one of these cases the material was said to have been dyed and boiled for more than 30 mins.

c = cases. d = deaths.

*Summary of Table XII.*

Bristles alone were suspected in	...	...	...	3	11	Bristle.
Bristles mixed with other raw materials do.	...	...	...	8		
Horsehair alone was suspected in	...	...	...	40	73	Horsehair.
Horsehair mixed with other raw materials do.	...	...	...	33		
Chinese material alone was suspected in manes	15 <sup>6</sup> ,	tails	2 <sup>0</sup> ,			
hair 8 <sup>3</sup> , and bristle 2 <sup>0</sup>	...	...	...	27 <sup>9</sup>	48 <sup>10</sup>	Chinese raw material.
Chinese material mixed with other raw materials ditto,	hair 13 <sup>5</sup> ,					
Yak 3 <sup>0</sup> , horsetails 1 <sup>0</sup> , bristles 2 <sup>1</sup> , hair and bristle 2 <sup>1</sup>	...	...	21 <sup>7</sup>			
Siberian material was suspected alone in hair	4 <sup>1</sup> ,	tails	3 <sup>1</sup>	7 <sup>2</sup>	20 <sup>6</sup>	Raw material from Russian Empire
Siberian material mixed with other raw materials ditto,						
bristles 1 <sup>1</sup> , hair 8 <sup>3</sup> , tails 1 <sup>0</sup> , Yak 3 <sup>0</sup>	...	...	13 <sup>4</sup>			
Russian material alone was suspected in tail	1 <sup>0</sup> ,	bristle	1 <sup>0</sup> ,		41 <sup>12</sup>	
hair 4 <sup>1</sup>	...	...	...	6 <sup>1</sup>		
Russian material mixed with other raw materials ditto,	hair 13 <sup>5</sup> ,	hair and bristle 1 <sup>0</sup> , bristles 1 <sup>0</sup>	...	15 <sup>5</sup>	21 <sup>6</sup>	
Australian material alone was suspected in hair	1 <sup>0</sup>	...	1 <sup>0</sup>			
Australian material mixed with other raw materials ditto,					3 <sup>0</sup>	Australian raw material.
Yak etc. 3 <sup>0</sup>	...	...	...	...		
English material alone was suspected in cow	2 <sup>1</sup> ,	horsehair	2 <sup>0</sup>	4 <sup>1</sup>		
English material mixed with other raw materials ditto, cow					7 <sup>2</sup>	English raw material.
and horsehair 1 <sup>0</sup> , cow hair 1 <sup>0</sup> , hair 1 <sup>1</sup>	...	...	3 <sup>1</sup>			
American material alone was suspected in bristle	1 <sup>0</sup> ,	hair	2 <sup>0</sup> ,		4 <sup>0</sup>	American raw material.
horsehair and cow hair 1 <sup>0</sup>	...	...	...	...		
American material mixed with other raw materials ditto,					10 <sup>3</sup>	
bristle 2 <sup>1</sup> , hair 8 <sup>2</sup>	...	...	...	...		
Indian material mixed with other raw material was suspected					1 <sup>1</sup>	Indian raw material.
in bristles 1 <sup>1</sup> (Indian)	...	...	...	...		
Native hair alone was suspected in	...	...	...	1 <sup>0</sup>	1 <sup>0</sup>	
Not stated	...	...	...	22 <sup>1</sup>	22 <sup>1</sup>	

The smaller figures indicate the fatal cases.

years' use, and the material was being put through a hand teasing machine, when the workman scratched the back of his hand, and a malignant pustule developed in that position.

From 1899-1907 bristles alone were said to be the source of infection in only three cases, bristles mixed with hair in eight cases, making 11 cases in which bristles were suspected against 73 in which horsehair was suspected. (Table XII and Summary.)

Horsehair alone was suspected in 40 cases, and mixed with bristles and other hair in 33 cases. Chinese and Russian (including Siberian) material was the most dangerous giving rise respectively to 48 and 41

cases. America is third with 14 cases. These figures are of little value unless we examine them in relation to the amount imported.

TABLE XIII.

*Relation between the weight and value of imports and cases  
of Anthrax 1899—1907.*

Material	Imports in 1902, tons, weight	Imports in 1902, value in thou- sands of £	Average number of cases of anthrax per annum	No. of tons of material to each case of anthrax	Value of mate- rial in thou- sands of pounds for each case of anthrax
Horsehair and Bristles	3708·75	876·5	9·3	398·79	94·25
Bristles	2046	626·5	1·2	1705	522
Horsehair	1662·75	249·75	8·1	205·2	30·8
Chinese raw material	1076	189	3	358·6	63
Russian & Siberian raw material	891·5	266·5	1·4	636·75	193
American raw material	129·5	17	·44	294·3	38·6
Australian raw material	101	17·5	·11	1010	175

Table XIII shows that (1) horsehair is more than eight times as dangerous as bristles; (2) that taking into consideration the quantities imported the risk of infection is greatest from Chinese and Russian raw materials.

Errors in the last table arise because it is impossible to estimate the amount of material of any particular country that passes through Great Britain without being manufactured. The exports of bristles are large, most going to the United States of America. In 1901 the value of imports of bristles from all countries was £527,691 of which £232,283 worth were re-exported. In the case of horsehair also the export trade is large, though in this case the exports contain probably a little English hair as well as re-exported foreign hair. In 1901 the imports of horsehair were valued at £165,702 and exports £82,211. A large proportion of the latter went to the U.S.A. Also raw material from one country is often shipped from a port in another country, and the material is then entered under the country to which the port belongs. Practical experience in this country, however, bears out my conclusions. Hair from China, Russia, and Siberia, and more rarely from America, being considered by English manufacturers the most dangerous. Similarly in France, Italy and Germany, raw materials from China, Russia, and Siberia are all considered more dangerous than other kinds.

Debray from observations at St Denis states that hair from China, Russia, Australia, and Siberia, are the most frequent sources of infection.

In some English factories and workshops isolated cases have occurred, in others repeated attacks follow one another. Incidence is not limited to any particular district, and an examination of the locality shows that cases of anthrax due to the manipulation of horsehair are distributed evenly with the number of people employed, the dangerous classes of hair being used very generally. In the woollen industries it is otherwise. Anthrax is common among workers in wool in the West Riding of Yorkshire especially in Bradford and Worcestershire. There dangerous classes of wool are commonly used. In the south of England and Scotland, anthrax is conspicuously absent from the woollen districts, as in these places only colonial and home-grown wools are manipulated. In the hide and skin industries most of the cases occur in Liverpool and London; and the Liverpool Urban Sanitary Authority have made anthrax notifiable for three years from 1907. For the three years ending 1906, 21 cases of anthrax, mostly among dock labourers, occurred in Liverpool, of these 20 were external and 14 died.

#### *Anthrax among animals.*

The accuracy of statistics of anthrax among animals must be doubted, as any animals that die suddenly are often said to have died of anthrax.

TABLE XIV.

*Gross number of animals attacked with Anthrax in various countries.*

Country	1902	1903	1904	1905	1906	Averages
United Kingdom ...	... 1032	1142	1589	1317	1233	1263
Germany ...	... 4852	4626	5959	6133	6226	5559
Italy ...	... 6099	4059	3946	3783	5039	4585
British India ...	... 28081	30715	?	?	?	29398
Russia :						
European Russia, Finland and North Caucasus ...	42423	47300	?	?	48783	46169
Asiatic Russia and Trans- Caucasus ...	6802	4200	?	?	2906	4636

TABLE XV.

*Number of premises in which cases of Anthrax occurred among animals.*

Country	1901	1902	1903	1906	Averages 1901—1903
France	416	395	491	416	434
Hungary	1974	2158	2754	—	2295
Sweden	224	218	179	—	207

From the figures given in Tables XIV and XV, it will be seen that anthrax occurs among animals practically all over Europe and Asia, and that in European countries it is most frequent (taking into consideration the area) in Italy and Hungary, and more frequent in Germany than in France, Sweden or England. Probably in France there are fewer cases among animals than in any other country, this may possibly be accounted for by the carrying out of vaccination under the Pasteur system.

In both the Russian and Indian Empires, anthrax is extremely common among animals.

In China anthrax among animals is said to be a rare disease, at any rate it is probable that it does not occur to the extent one would expect. Trustworthy information is entirely lacking, but two events indicate that anthrax does occur. In the Tibetan mission a few years ago, anthrax among the Yaks reduced the number from 3000 to 1450. Also some Australian cattle imported into China all died of anthrax at Wuchow.

In Australia the incidence of anthrax is small, precautions are taken to prevent the spread of the disease and to secure proper disposal of the carcase.

In the United States of America, in the Argentine and Uruguay, consular reports mention scattered outbreaks of anthrax from time to time, but no definite figures are obtainable. Similar precautions are taken as in the case of Australia.

From 1903-1905, 92,113 bovines were reported to have died of anthrax in India and Burma.

Tables XVI and XVII give the class of animal attacked and the incidence.

Among cattle the incidence is highest in Italy and Germany and low in England and France.

Among horses the incidence is much the highest in Italy, England again being low; but among swine the English and Italian figures closely approximate, while the German figures are so low as to make one suspect that many cases pass unreported, considering the half wild condition of many German swine this is not improbable.

In certain countries the incidence is greatest in the hot summer months; this is well shown by the curve in the diagram on page 310 which shows that in Russia in 1906, there was no change in the incidence of anthrax until March, when the number of cases rapidly increased, reaching a maximum in July; after which there was a fall until in November and December the figures reached the level of the

early months of the year. An examination of the statistics in any recent year will show a similar result, and Dr Legge states that the same seasonal variation may be observed in Germany. On the other hand in Great Britain we have fewer cases in the warmer months of

TABLE XVI.

*Cases of Anthrax among animals.*

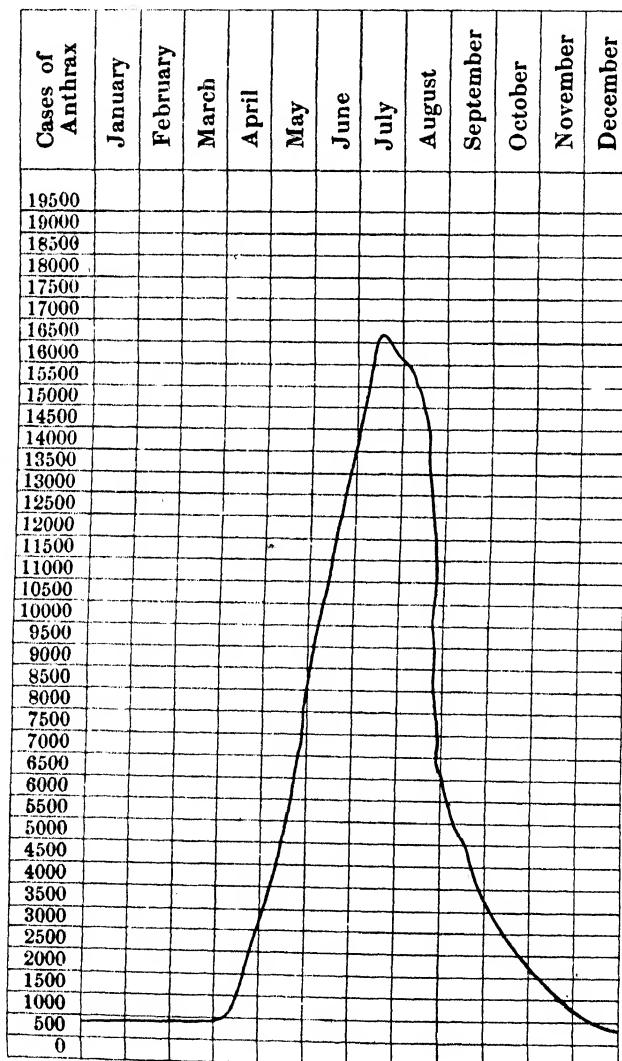
Country	Animal	1902	1903	1904	1905	1906	Average no. affected per annum
United Kingdom	Cattle	746	807	1115	1001	937	921
	Horses	44	51	47	53	?	49
	Sheep & Goats	50	48	62	53	83	59
	Swine	192	234	365	210	213	243
Germany	Total	1032	1142	1589	1317	1233	1272
	Cattle	4003	3990	4571	5308	5390	4652
	Horses	134	150	177	172	183	163
	Sheep	620	339	1111	509	502	616
	Goats	8	11	12	13	14	12
	Swine	87	136	88	131	137	116
Italy	Total	4852	4626	5959	6133	6226	5559
	Cattle	?	1294	1311	1195	1444	1311
	Horses	?	111	67	32	97	77
	Sheep	?	2275	2317	1339	} 3357	2453
	Goats	?	262	140	124		
	Swine	?	117	111	93	141	115
	Total	?	4059	3946	2783	5039	3956

TABLE XVII.

*Incidence of Anthrax per 100,000 of cattle, horses and swine.*

Country	Number of cattle in country indicated	Average number of cattle affected with anthrax	Incidence of anthrax per 100,000 cattle	Number of horses in country indicated	Average number of horses affected with anthrax	Incidence of anthrax per 100,000 horses	Number of swine in country indicated	Average number of swine affected with anthrax	Incidence of anthrax per 100,000 swine
United Kingdom	11,961,955 (1907)	921	7·6	2,110,024 (1907)	49	2·3	3,580,740 (1907)	243	6·7
Germany	18,939,692 (1900)	4652	24·5	4,195,361 (1900)	168	3·9	16,807,014 (1900)	116	.69
Italy	5,000,000 (1900)	1311	26·2	741,739 (1900)	77	10·3	1,800,000 (1900)	115	6·4
France	14,673,810 (1901)	804 (1892)	5·4	2,926,382 (1901)	?	?	6,758,198 (1901)	?	?

July, August, and September. From 1899-1904 (six years) there were 4250 cases of anthrax among animals in Great Britain. Of these 1114 occurred in the first quarter, 1166 in the second quarter, in the third 850 and the fourth 1120. In countries where cases of anthrax arise largely from actual contact with affected animals, and industrial conditions are not prominent, it will be found that incidence in man varies with the incidence in animals; thus in Italy from 1892 to 1896



(five years) there were 11,102 cases of human anthrax. Of these 1482 occurred in the first quarter, 1398 in the second quarter, 5286 in the third, and 2936 in the fourth. In Italy as in Russia incidence in animals is greatest in the hot summer months. An explanation of this seasonal variation may be found in the changes in temperature, soil, and vegetation.

An animal affected with anthrax while still alive sheds into the air by the bloody discharges, and by the excretions from mouth, nose, and bowel, countless bacilli which readily spore. These spores, extremely resistant, may remain potent for long periods. Under suitable conditions of temperature which occur at certain seasons of the year, these spores develop as they can grow on organic matter in nature. Koch found that excellent media for growth could be obtained from seeds rich in starch, such as barley, wheat, grass, and potato and turnip juice; water in which hay has been allowed to decay, if neutralized and made slightly alkaline, is a suitable medium. Chalk in the soil would tend to make the medium alkaline.

Heusinger found that anthrax was most commonly met with on the salt grass steppes, river valleys, on round little lakes, and on lands rich in organic matter liable to putrefy.

Billing states that districts where anthrax is most prevalent contain profuse vegetation, are moist compared with surrounding districts, often liable to be flooded, but drying out considerably in the summer, often exposed to the full action of the sun, and protected from cooling winds.

In the Prairie States the most marked spots are the cups in the high prairie land more or less full of stagnant water, liable to flood in spring, while the surrounding country is parched and dry. In August and September green verdure grows around the cups where the water has overflowed, and the temperature is warm enough for the development of the bacillus among the roots of the grass.

Koch, from observation of the principal anthrax districts in France (banks of the Loire, Oise, Aisne, Saone, and the marshy low-lying country of the Gironde), in Germany (banks of the Danube, swampy sides of the Bavarian Alps, valleys of the Elbe, Mulde, Saale, Bude) districts all liable to floods, concludes that the favouring conditions of soil are (1) fairly impervious soils of chalk, marl or clay; (2) sandy soil but only where the sand lies in a thin layer on impervious ground, and where it is intricately mixed with the decomposing animal and vegetable matter; (3) peaty soils especially rich in organic matter and mineral

substances, such as prairies, steppes, moor, and marsh land. The essentials for any particular place to become a more or less permanent source of infection are (*a*) *Bacillus anthracis*, (*b*) a suitable soil, at times moist at others drying out, profuse vegetation and decay, (*c*) a suitable temperature. The *B. anthracis* develops best at 35° C. and multiplication may take place anywhere between 12° C. and 45° C., while cooling below freezing does not kill. Spores are best produced at 35° C. but sporulation may take place between the limits 18° C. and 42° C.

That such a district would remain a permanent source of infection is improbable since the bacilli would become attenuated and eventually die out; but from time to time a fresh supply is brought in by the discharges from animals infected from grazing on the place.

If anthrax bacilli are kept at 42° C. for eight days, they lose the power of sporulation and become less virulent, even when grown at a lower temperature, only regaining it after passage through the body of a susceptible animal.

The fact that in Great Britain there is no increased incidence of anthrax during July and August, as in most other countries, points to the absence of those special conditions which are necessary for the germination of the spores into bacilli and for the multiplication of these. However in most countries such districts as those above described do exist, and on them anthrax certainly flourishes; here too may occur the contamination of the hides and hair and wool, owing to the dirt and sand, containing the spores of anthrax, which become adherent to the long hair and fleeces, as these are trailed upon the ground.

#### SUMMARY.

At the commencement of this paper it was pointed out that anthrax has been associated with the manipulation of hair and other raw animal materials for at least a century and a half, but that it has only been studied from an industrial point of view during the last 30 years, with the result that in 1895 cases of anthrax, occurring in workshops and factories, became notifiable. From that time to the end of 1907, over 500 cases have been brought to the notice of the Home Office. Beside these cases of industrial anthrax many others arise, chiefly from contact with animals or animal carcases affected with anthrax; these are not notifiable in this country.

The proportion of industrial to non-industrial cases of anthrax is about 4 to 3.

An analysis of cases of anthrax notified between 1898 and 1907 shows :

- (1) A progressive increase in the number of cases in the woollen and worsted industries, probably due to the increased use of dangerous classes of wool.
- (2) A progressive increase in the rate of mortality, due to the larger number of internal cases among manipulators of wool.
- (3) A lower rate of mortality among workers on horsehair and bristles than in other industries.
- (4) A high rate of mortality in the group of miscellaneous industries in which anthrax is not usually suspected, the diagnosis being then made too late for effective treatment.

In order to make an early diagnosis it is essential for anthrax to be suspected from the nature of the employment.

Among bristle and horsehair workers :

- (1) Anthrax is more fatal to the women than to the men.
- (2) The occupational risk is greater for the men than for the women, because the former are more often employed in the earlier, more dangerous manipulations.
- (3) The occupational risk is greater and the mortality higher among the manipulators of horsehair than in brushmaking.
- (4) Horsehair is from 8 to 10 times as likely to give rise to cases of anthrax as are bristles, and the risk of infection is greatest from Chinese, Russian and Siberian raw materials; this is confirmed by actual experience not only in this country but abroad.
- (5) The risk to workers on horsehair is greater than the risk to workers on wool.

The nature of the lesion varies considerably with the industry, inhalation of spores being uncommon in any but the woollen industry, though internal cases undoubtedly occur among horsehair manipulators. This is not surprising as many of the processes in these industries are similar.

The position of the pustule varies slightly with the occupation ; for example, pustules on the neck are most frequent among hide and skin workers, due to the frequent carrying of skins on the shoulder. Malignant pustule is most common on the exposed parts, and of the latter those less frequently washed, as the neck and face. The infection is in most cases by the nails, which harbour the dust containing anthrax spores.

In agricultural cases lesions on the upper extremity are most common.

The mortality varies with the situation. Difficulty of operation, frequent absence of the diagnostic signs of local necrosis, looseness of the cellular tissues increase the mortality.

In all countries except Great Britain agricultural anthrax is the most common, and consequently there is a close relation between the number of cases of human and animal anthrax.

Variations in the prevalence and mortality of anthrax among human beings occur in different countries, and may to some extent be accounted for by natural constitution and environment.

There has been a progressive increase of cases in Italy, but a decrease of the death-rate, roughly corresponding to the introduction of Sclavo's serum treatment.

French statistics, as do the English, emphasize the greater risk to horsehair workers, though the risk and mortality in Great Britain compare very favourably with those in France, and still more so with those in Germany.

The danger of infection from anthrax in the manipulations of animal hair and bristles depends on the origin, kind and cleanliness of the materials, and the processes they have to undergo.

Bristles as imported vary immensely, some being well cleansed and prepared, others in an extremely dirty state, but they are always in a far more prepared state than the horsehair.

The long tail horsehair, being more valuable, is better cleansed and less likely to cause infection than the shorter mane horsehair.

Cases of anthrax occur in practically all the processes to which hair is subjected from its time of arrival even to the finished products. Further, incidence is greatest among workers in short hair as brush-makers, and in the earlier processes, i.e. when the hair is in a less prepared state.

Infection may be carried in clothes or nails to people outside by workers.

Anthrax spores may retain their vitality for years on hair and other materials.

The dangerous classes of hair are very generally used. This is not so in the woollen and worsted trades.

Anthrax is common among animals all over Europe and Asia, and in most countries there is an increased incidence during the hot summer

months. It is probable that in certain districts special conditions exist which make them permanent centres of anthrax infection. Here too hides and hair from contact with the soil may possibly become infected without the animals actually contracting anthrax.

*Note.*

References to the Literature will be found at the conclusion of the second part of this paper which will appear in the next number of this *Journal*.

ON HETEROLOGOUS AGGLUTININS MORE PARTICULARLY  
THOSE PRESENT IN THE BLOOD SERUM OF CEREBRO-  
SPINAL FEVER AND TYPHUS FEVER CASES.

By W. JAMES WILSON, B.A., M.D. (R.U.I.), D.P.H. (Camb.).

*Lecturer on Hygiene, Queen's University, Belfast.*

(*From Pathological Laboratory, Queen's University, Belfast, and  
Hygienic Institute, Berlin.*)

(1 Text Figure.)

IN previous papers Professor Symmers and myself have called attention to the presence of agglutinins for the typhoid bacillus, the colon bacillus, and a bacillus of the alcaligenes class in the blood serum of patients suffering from cerebro-spinal fever from whose lumbar puncture fluid and in some cases from whose blood the *Micrococcus intracellularis meningitidis* (Weichselbaum) had been cultivated.

The results obtained were so anomalous that it appeared to me desirable to continue the investigations and to find if our work could be brought into line with that of others in this special field. In consequence an extensive literature of the subject of Agglutination has been consulted and such facts as seemed to belong to the same class as ours noted.

In the present paper the subject matter has been arranged in the following order.

I. A brief recapitulation of the main points of the previous results obtained by Professor Symmers and myself.

II. A rapid survey of our knowledge of agglutination and in this way an endeavour is made to find the proper setting of our results.

III. In the concluding portion some further original observations are recorded.

## PART I.

*Agglutination of bacilli of the typhoid, colon and alcaligenes group by the blood serum of cases of cerebro-spinal fever.*

Emulsions of agar cultures of the microorganisms were made in normal saline solution. The microscopic method of examination was used throughout and the results were recorded at the end of one or two hours.

*Agglutination of the B. typhosus.*

Seven out of 21 samples of the serum of cerebro-spinal fever patients examined "clumped" the *B. typhosus* in a 1 in 50 dilution, and of these seven three still gave a positive result with a 1 in 200 dilution and one with a 1 in 400 dilution.

*Agglutination of the B. coli.*

A "flaginac"<sup>1</sup> colon bacillus isolated from the faeces of a cerebro-spinal fever case was used as the test organism and 18 samples of blood serum were examined. In 16 of them the results were negative and in two positive with a 1 in 50 dilution of the serum. One of these cases still gave a positive reaction with a 1 in 400 dilution.

*Agglutination of a bacillus of the alcaligenes class.*

This bacillus is actively motile, forms a uniform emulsion in normal saline solution and in its growth on media resembles the *B. faecalis alcaligenes*. It was isolated from Belfast tap water. It was formerly called by us the *B. Grosvenor* as it was obtained from a house on Grosvenor Road; we now call it the *B. aquatilis alcaligenes*, and, bearing this name, it can be obtained from Professor Král, Prague.

We have examined altogether 184 different samples of the serum of cerebro-spinal fever cases and of these 153 gave a positive reaction with a dilution of 1 in 50. Of the 31 cases giving negative results 10 were found to be positive on a second examination, one on a third examination, three were examined several times and were still found negative, the remaining 17 were examined only once and it is probable that a later

<sup>1</sup> An organism producing fluorescence in glucose neutral red shake cultures, acid and gas in lactose broth, indol in peptone water, acid and clot in litmus milk.

examination of many of these would have given positive results ; in the case of others the examination was made late in convalescence when the agglutinins which were probably originally present had disappeared.

In round numbers 90% of the cases of cerebro-spinal fever agglutinated the *B. aquatilis alcaligenes* in a dilution of 1 in 50. In most cases agglutination still occurred with a 1 in 100 and higher dilutions. In 16 cases the blood serum gave marked "clumping" within an hour to dilutions of 1 in 1000, and, further, we found that one of the 16 agglutinated in 1 in 1400, 2 in 1 in 1500, 4 in 1 in 1600 and 4 in 1 in 2000 dilutions respectively. We have shown that it is possible to remove the agglutinins from the serum by saturation with the *B. aquatilis alcaligenes*, whilst saturation with the *Meningococcus*, *B. typhosus*, *B. coli communis* or *B. faecalis alcaligenes* (Král) leaves them intact. These results were controlled by the examination of the blood serum of healthy adults and of patients suffering from various infectious and general diseases.

Of the 31 specimens of the serum of normal adults examined 3, i.e. 9·6%, gave a positive reaction in a 1 in 50 and all a negative reaction in a 1 in 100 dilution of the serum. Of 137 samples of serum taken from patients suffering from various diseases 35 gave a positive and 99 a negative reaction in a 1 in 50 dilution, 3 a positive reaction in a 1 in 100 dilution. In higher dilutions than 1 in 100 all these cases gave negative results.

In round numbers 22% of the controls gave a positive result in a dilution of 1 in 50.

## PART II.

### *Main facts in the Literature of Agglutination particularly those having reference to heterologous agglutination.*

The discovery that the blood serum of an animal which had been immunised against certain microbes, e.g. *B. typhosus*, *B. cholerae*, etc., possessed a strong agglutinating action on the infecting microbe, soon proved to be a fact of great scientific and practical value. Gruber and Durham (1896) first applied "agglutinins" for the purpose of distinguishing microorganisms, whilst Grünbaum (1896) and Widal (1896) showed that in it clinical medicine had obtained a new method of diagnosing disease.

It was early recognised that the test was not a qualitative but a quantitative one. Stern (1903) as the result of an examination of the blood serum of normal adults showed that the reaction to be of value

must be obtained in a dilution of at least 1 in 40 of the serum. When the blood serum of typhoid fever patients was examined with regard to its agglutinative action on other bacilli, e.g. *B. coli*, *B. enteritidis*, it was found that these microbes also at times were agglutinated.

Some observers interpreted this fact as indicating that in typhoid fever not only was there an infection with the Eberth-Gaffky bacillus but also with certain intestinal microbes. The results obtained by observing the effect of the inoculation of animals with pure cultures showed that the increase of the agglutinins for *B. coli* in the blood of the animals which had been inoculated with the *B. typhosus* was due to the stimulating effect of the *B. typhosus* alone and in no way indicated a secondary infection with the *B. coli*. In this enquiry Pfaundler (1899), Durham (1900), Wassermann (1903), Jatta (1900), Jürgens (1903), Drigalski and Conradi (1903) took a prominent part. These researches impugned the specificity of the test. Pfaundler (1899) brought the knowledge at that time to a focus by saying that when an animal is immunised against an organism it produces in its serum not only agglutinins for this organism but also for nearly related microbes. In his own words specific agglutinins and group agglutinins are produced. Subsequent work showed that the agglutinative effects of an immune serum were increased not only for nearly related organisms but also for those which stood further away in a botanical classification. Wassermann (1903) brought the new facts into line concluding that in immunity primary agglutinins or hauptagglutinins are produced which act on the infecting organism and secondary or partialagglutinins which act on other organisms. This secondary or indirect action was called mitagglutination. The formation of partialagglutinins varied in different experiments. The particular strain of microorganism used, the species and also the individual peculiarities of the animals seemed to be factors in this variation.

Durham (1900) formulated a theory with regard to agglutinins which corresponded with Ehrlich and Morgenroth's discoveries as regards immune bodies. Like the latter, agglutinin was not to be considered as a single substance but consisted according to Durham of numerous single agglutinins which corresponded to the components of the agglutinogen substance of the bacteria. If one indicated the agglutinins with the letters, *A*, *B*, *C*, etc. and the agglutinogens with *a*, *b*, *c*, etc. the result of the possession of a number of similar agglutinogens was manifested in the phenomenon of mitagglutination. An example will make this clearer. The *B. typhosus* has, let us suppose, as its components *a*, *b*, *c*, *d*, *e*, the *B. enteritidis* *c*, *d*, *e*, *f*, *g*, *h*; the corresponding

sera possess the agglutinins *A, B, C, D, E*, and *C, D, E, F, G, H*. Typhoid immune serum works with all its single agglutinins *A, B, C, D, E*, on the corresponding components of the *B. typhosus* and gives therefore a maximal effect, while on *B. enteritidis* only the agglutinins *C, D, E* come into action and therefore mitagglutination is demonstrated only in low dilutions of the serum.

Clinical experience showed that in cases of jaundice very often the blood gave a positive Widal reaction with the *B. typhosus*. This phenomenon is explained as being due to infection of the biliary passages with either the *B. typhosus* itself or organisms which cause the formation of partial agglutinins for the Eberth-Gaffky bacillus.

In Weil's disease Eckardt (1902) and Zupnik (1902) found that the blood serum agglutinated the *B. typhosus*. In two cases of Eckardt's the reaction was positive even in a dilution of 1 in 1000. Eckardt regarded Weil's disease as a special form of infection with the *B. typhosus*; however Jäger's (1892) view that Weil's disease is a distinct entity and that the infecting microorganism is a pleomorphic member of the *proteus* group has received support from other investigators.

At this point some observations and experiments of Lubowski and Steinberg (1904) may be appropriately mentioned. These authors had two cases of mixed infection with *B. proteus* and *Staphylococci*. In both cases the infection arose from a suppurative otitis media. In the first case the blood serum of the patient agglutinated the *B. typhosus* and the *B. proteus* in dilutions of 1:40 and 1:80 respectively. In the second case the blood serum agglutinated the *B. proteus* isolated from the first case and the *B. typhosus* in dilutions of 1 in 320 and 1 in 40, later in dilutions of 1 in 2500 and 1 in 80 respectively. Thus there was an increase of the agglutinins during the course of the illness. Inoculation with Cholera bacilli and Streptococci was found to cause no production of agglutinins for the *B. typhosus* in the serum of the animals but when *Staphylococci* were employed the serum agglutinated the *B. typhosus* in a dilution of 1:640.

The saturation experiments of Bordet (1899), Eisenberg and Volk (1902) showed that in normal serum there are agglutinins for different organisms which can be removed by the corresponding organism and that this process leaves the agglutinin content for the other organisms unimpaired.

Another cause for secondary agglutinins in the blood is mixed infection. Castellani's (1902) application of the specific absorption

method appeared to solve the question whether the secondary agglutinins present in a blood serum were due to mitagglutination or to a mixed infection. Castellani claimed that saturation of the serum with the infecting microorganism removed not only its own agglutinins but also the partial or secondary agglutinins, whereas the latter remained unaltered if they were due to infection with their corresponding bacillus.

No doubt Castellani's experiment gives accurate results in many cases, especially those in which infection with typhoid and para-typhoid bacilli are concerned; still the experiments of Posselt and Sagasser (1903), Hetsch and Lentz (1903), Ballner and Sagasser (1904), Park and Collins, Symmers and Wilson (1907) seem to minimise its worth.

Posselt and Sagasser (1903) showed that in immunising there is not only an increase in the amount of agglutinins for the organism used but also of secondary or nebenagglutinins which act on other organisms. These nebenagglutinins as regards absorption behave like the special agglutinins in cases of mixed infection or as the agglutinins in normal serum. An example from their paper shows that sometimes these nebenagglutinins may be increased to a high degree. Thus the serum of a guinea-pig immunised against the *B. typhosus* and of a titre of 1:12000 for this bacillus had nebenagglutinins for *B. cholerae* 1:4500 and for *B. dysenteriae* 1:4000.

Hetsch and Lentz (1903) demonstrated by absorption with genuine cholera bacilli and cholera-like vibrios, the specificity of the agglutinins in normal horse serum and in that of an animal immunised against the *B. cholerae*. Saturation with *B. cholerae* diminished the agglutinins for this organism whilst the nebenagglutinins remained either the same or were but slightly diminished.

Ballner and Sagasser (1904) showed that a homologous bacterial species can withdraw from an immune serum only its own agglutinins but not the nebenagglutinins which act on other bacteria: also a heterologous bacterial species binds only its own partial agglutinins and no other portion of the total agglutinins, so that for this reason Ballner and Sagasser conclude that the absorption of agglutinins through homologous and heterologous microorganisms must be regarded as a strong specific reaction. Examples given in Ballner and Sagasser's paper show that the nebenagglutinins are at times markedly increased, that inoculation with the *B. tetani* and *B. pneumoniae* of Friedlander lead to the formation of few hauptagglutinins but numerous neben-agglutinins.

## Heterologous Agglutinins

322 and Collins (1903) showed (1) that the group agglutinins may  
    mously increased, (2) that injection of bouillon alone may  
Par the agglutinins in a horse's serum, (3) that the absorption  
increase mply proves that when one variety of bacteria removes all  
method s for a second the agglutinins under question were not  
agglutinins that second variety.  
produced by (1908) has recently shown that inoculation with various

Collins (1908) (e.g. enzymes, yeasts, simple salts containing  
non-bacterial bodies or phosphorus atom) leads to the formation of  
either a sulphur very bacilli.

agglutinins for dysentery as *B. typhosus* itself or *B. coli*. Theory of Immunity implies the

It is well known that Ehrlich's theory affords a body and  
presence of protective substances already existing in the blood  
that in the immunising process these are increased in amount. It's

How the agglutinins of normal serum come into existence is obscure.  
The work of Grünbaum (1897), Müller (1901), Pfaundler, Kraus and  
Löw (1897) and Schumacher (1901) shows that the agglutinins for  
*B. coli* and *B. typhosus* are either absent or present in only very small  
amounts in the blood of young children and animals. In other words  
the agglutinating power of normal serum is not inborn but is produced  
during life. One may suppose a process of immunisation to be going on  
continually, the organisms in the intestine causing the formation of  
agglutinins in the blood. It may be noted that Fraenkel and Otto  
(1897) succeeded in increasing enormously the agglutinins in dog's  
blood by feeding the animals with large doses of typhoid bacilli.

The explanation of the fact that the blood serum of Europeans con-  
tains specifically absorbable agglutinins for the *B. cholerae* and the *B.*  
*pestis* may be that these agglutinins are partial agglutinins or nebenagglu-  
tinins caused by the action of unknown saprophytic intestinal organisms.

It is not unlikely that saprophytic organisms resembling pathogenic  
ones are in the alimentary tract and that hauptagglutinins are pro-  
duced for these organisms and nebenagglutinins for their pathogenic  
relations. It is well known that agglutinins can be formed by inoculation  
with the simplest saprophytes, e.g. *B. mesentericus*.

Whether the agglutinins of normal and immune serum are identical  
is undecided. Ehrlich's theory supposes that immunisation is only the  
normal production of receptors carried to excess.

Landsteiner and Reich (1908) in a recent paper have pointed out  
that the haemagglutinins of normal serum are more easily removed than  
the homologous agglutinins of immune serum by shaking the serum  
with casein. Normal agglutinins were also found to be more heat

labile than immune ones. They also showed that each single agglutinin of normal serum has an affinity for a number of different corpuscles and that the amount of this affinity varied in different cases. They remark "in the language of Ehrlich our results tend to the view that the haptophorous groups of normal agglutinins are so composed that they can react with the different receptors of very many blood corpuscles but in unlike degree. But according to Ehrlich's theory each haptophorous group ought to react with only a single fixed kind of receptor." They also found that when an animal was inoculated with the blood corpuscles of another species not only the homologous agglutinins but also the *neben-agglutinins* which act on the blood corpuscles of other species increase. These results correspond with those of Posselt and Sagasser and Hetsch and Lentz.

As a rule the homologous agglutinins relatively preponderated but to this in two cases there were striking exceptions, in which during the immunising the heterologous agglutinins became very high and were distinctly higher than the homologous ones.

The very definite results obtained by absorption experiments point to the presence in normal serum of specific agglutinins for the different bacteria. The recent work of Bürgi (1907), Mamlok (1908) and Hirschfeld (1907) has furnished results rather opposed to this view and which find their simplest explanation in the assumption of a single normal agglutinin which acts on all bacteria and blood corpuscles.

Bürgi (1907) found (1) that if a normal serum agglutinated one bacillus strongly or weakly, it had a proportionately strong or weak effect on other bacteria, (2) that animals could be arranged in a series according to the action of their blood serum on the most different bacteria. Cattle serum had the strongest whilst guinea-pig serum had the weakest agglutinative effect.

From this review which I shall conclude with a quotation from Paltauf it can be seen that the results of Posselt and Sagasser, Hetsch and Lentz, Ballner and Sagasser, and Park and Collins are along the same lines as those obtained by Professor Symmers and myself.

Paltauf (1904) says the results of Posselt and Sagasser as well as those of Hetsch and Lentz go to show that in the immune serum of animals as well as in that of sick men and women heterologous agglutinins exist which have no binding groups for the infecting bacteria but are as specific as regards absorption as those developed in a mixed infection. They must therefore be distinguished from partialagglutinins or mitagglutinins. They can be designated as "heterologous *neben-*

"agglutinins" or more briefly as *nebenagglutinins*. For their formation the views held regarding partial agglutinins do not apply.

To explain their origin one must assume that besides the receptors (homologous) that have binding groups fitted to the agglutinogens of the infecting organism other closely related receptors are set free. In part they would appear to be normal agglutinins whose production through an adequate stimulus is increased.

Perhaps one may conceive of them being fixed to the same protoplasm as that possessing the homologous receptors.

We have moreover a few examples of a receptor apparatus being stimulated to secretion through the irritation of a non-homologous haptophorous group. In this connection one may recall Verney's observations on the mutual influence of consecutive immunisations which showed that immunisation with typhoid bacilli affected the agglutinins for the *B. coli*.

Most pertinent are the observations of Obermeyer and Pick (1904) regarding the formation of a heterologous precipitin after it had been once formed through homologous immunisation and then a foreign protein injected. They saw the precipitins which had been formed as the result of inoculation several months previously with cattle serum and which had disappeared reappear on the injection of horse albumoses, and in such amounts that they could not be regarded as partial precipitins.

We may also mention that v. Dungern (1903) observed among rabbits injected with majaplasma one the serum of which precipitated not only majaplasma but also octopus plasma.

The explanation of the production of heterologous agglutinins may be that infection with certain germs leads to an alteration of the bacterial flora of the intestine. As the result of this secondary auto-infection, along with the agglutinins for the primary infecting organism, agglutinins are formed for the intestinal microorganisms also.

### PART III.

The explanation of the presence of these agglutinins for the *B. typhosus*, *B. coli* and *B. aquatilis alcaligenes* in the blood serum of patients infected with Weichselbaum's Meningococcus is either that there is a mixed infection or that the agglutinins are heterologous *nebenagglutinins*, the latter term being used to indicate agglutinins which are

shown by the absorption test to be distinct from the meningococcal agglutinins.

Against the idea of a mixed infection are the facts—(1) that no bacilli resembling the *B. typhosus* or the *B. aquatilis alcaligenes* were ever obtained from the blood, urine or organs of the cases though these were frequently examined by cultural methods, (2) that we got similar results with the blood serum of Scottish cases—we may recall the fact that the *B. aquatilis alcaligenes* was isolated from Belfast tap water, (3) that, as will be shown later, immunisation of animals with the Meningococcus increases the agglutinins for the *B. aquatilis alcaligenes*, (4) that the phenomenon was observed in such a large number of cases as to render the hypothesis of a double infection extremely improbable.

In this Part experiments were carried out to determine:

- (1) the course of development of the agglutinins for the Meningococcus, and for the *B. aquatilis alcaligenes* in a case of cerebro-spinal fever;
- (2) the effect of heat on these agglutinins;
- (3) with what component of the serum the agglutinins for the *B. aquatilis alcaligenes* are bound;
- (4) whether immunising an animal with the Meningococcus increased the agglutinins for the *B. aquatilis alcaligenes* in its blood and *vice versa*.

*Development of agglutinins during the course of  
Cerebro-spinal Fever.*

The blood serum of a girl 8 years of age was examined at short intervals as to its agglutinative action on a strain of the Meningococcus and on our culture of the *B. aquatilis alcaligenes*. The case was under observation from 30 hours after the onset of the disease until 47 days later at which time the patient left hospital completely cured.

The experiments were carried out at room temperature with emulsions in normal saline solution of agar and ascitic agar cultures of the *B. aquatilis alcaligenes* and Meningococcus respectively.

The results were recorded at the end of 2 hours. In Table I +++ indicates very large clumps, ++ clumps of moderate size, + small clumps.

From these results we see that in this case there was a rapid increase in the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes* during the fourth day of the disease, that these both attained

### *Heterologous Agglutinins*

TABLE I.



#### EXPLANATION OF CHART.

On the base line are marked the days of the disease; on the ordinates the dilutions of the serum at which agglutination was still present. On the ordinates is also marked the evening temperature of the patient.  
 x — x indicates the curve of the agglutinins for the Meningococcus (strain G) and this was not agglutinated by normal serum in a dilution of 1 in 2.  
 x —— x indicates the curve of the agglutinins for the *B. aquarilis* *deletigenes* and as this bacillus is agglutinated by normal serum with a 1 : 35 dilution a line ..... is drawn through this part of the Chart.

*Heterologous Agglutinins*

their maximum on the eighth day, after which date the meningo-coccal agglutinins declined slowly and the agglutinins for the *B. aquatilis alcaligenes* more rapidly.

When the agglutination results for these two organisms are plotted out in the form of curves it is seen that the latter are to a certain extent parallel. From many other experiments we have an impression that as a general rule a serum possessing a high content of agglutinins for the Meningococcus has also a high content for the *B. aquatilis alcaligenes*. This however is by no means invariably the case as can be seen in Table II.

TABLE II.

Name of patient	Date	Day of disease	Meningococcus strain G								<i>B. aquatilis alcaligenes</i>			
			5	10	20	40	50	60	80	20	20	40	60	100
S.	8/2/09	3rd	+++	+++	+++	++	+	-	-	-	-	-	-	-
	11/2/09	6th	+++	+++	+++	++	+	-	-	-	-	-	-	-
	18/2/09	13th	+++	+++	+++	+++	++	+	-	+++	+++	+	-	-
	11/3/09	34th	+++	+++	+++	+++	++	+	-	++	-	-	-	-

Table II also shows that agglutinins for the *B. aquatilis alcaligenes* may develop during the disease and yet a single examination of the blood may show no indication of them.

With reference to the agglutination of Meningococci it may be of interest to record here a result we obtained which showed that a great difference as regards agglutination exists between strains of Meningococci obtained from epidemic and sporadic cases.

*Agglutination of Meningococcus (Strain D, epidemic).*

	10	20	40	100	200	300	400	600	800
Flexner's Serum No. I	+++	+++	+++	+++	+++	+++	++	-	-
Ruppel's Serum No. II	+++	+++	+++	+++	++	+	-	-	-

*Agglutination of Meningococcus (Sporadic Strain).*

	10	20	40	100	200	300	400	600	800
Flexner's Serum No. I	-	-	-	-	-	-	-	-	-
Ruppel's Serum No. II	-	-	-	-	-	-	-	-	-

Effect of heat on the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes*.

This was tested by noting the effects of heating a specimen of a cerebro-spinal fever patient's serum and a specimen of a commercial antimeningococcal serum at 60° C. and at 65° C. for 10 minutes.

The results are represented in Table III in which +++ denotes large, ++ moderate sized and + small clumps, whilst - indicates absence of agglutination.

TABLE III.

## Meningococcus (Strain G).

	Dilutions of serum								
	2	5	10	20	30	40	60	100	200
<b>Patient's Serum :</b>									
Before ...	+++	+++	+++	+++	++	+	-	-	-
After 10 mins. at 60° C.	-	-	-	-	-	-	-	-	-
After 10 mins. at 65° C.	-	-	-	-	-	-	-	-	-
<b>Antimeningococcic Horse Serum :</b>									
Before ...	+++	+++	+++	+++	+++	+++	+++	+++	++
After 10 mins. at 60° C.	-	-	-	-	-	-	-	-	-
After 10 mins. at 65° C.	-	-	-	-	-	-	-	-	-

*B. aquatilis alcaligenes.*

	Dilutions of serum								
	20	40	60	100	200	300	400	500	600
<b>Patient's Serum :</b>									
Before ...	+++	+++	+++	+++	+++	+++	++	++	-
After 10 mins. at 60° C.	+++	+++	+++	++	-	-	-	-	-
After 10 mins. at 65° C.	-	-	-	-	-	-	-	-	-
<b>Antimeningococcic Horse Serum :</b>									
Before ...	+++	+++	+++	+++	+++	++	+	-	-
After 10 mins. at 60° C.	+++	++	+	-	-	-	-	-	-
After 10 mins. at 65° C.	-	-	-	-	-	-	-	-	-

It is evident from above Table that heating the serum for 10 minutes at 60° C. completely destroys the agglutinins for the Meningococcus but only partially those for the *B. aquatilis alcaligenes*, and that heating for 10 minutes at 65° C. totally destroys the agglutinins both for the Meningococcus and for the *B. aquatilis alcaligenes*.

With what component of the serum are the agglutinins for the *B. aquatilis alcaligenes* bound?

The original titre of a serum was as follows:—

100	500	800	1000
+++	+++	+++	++

Equal volumes of this serum and of a saturated watery solution of ammonium sulphate were taken. A precipitate of globulin was formed. The mixture was centrifugalised and the supernatant fluid was found to be devoid of agglutinative effect. The precipitate was dissolved in normal saline solution and the volume made up to that of

the original volume of the serum. This solution was found to agglutinate the *B. aquatilis alcaligenes* in the following manner:—

100	200	500	800	1000
+++	+++	+++	++	+

To discover what fraction of the agglutinin was bound up with that portion of the globulin known as the "euglobulin" and what with the "pseudoglobulin" component, the following experiment was performed.

2·5 c.c. serum, 5·75 c.c. distilled water and 4·25 saturated watery solution of ammonium sulphate were mixed together so that there were 3·4 c.c. of saturated watery solution of ammonium sulphate in 10 c.c. After standing two hours at room temperature the mixture was centrifugalised. The supernatant fluid contained the "pseudoglobulin" fraction. The precipitate (euglobulin) was washed with water containing 3·4 c.c. saturated ammonium sulphate solution per 10 c.c. The "euglobulin" was dissolved in normal saline solution and the volume made up to 2·5 c.c.

The agglutination titre of the pseudoglobulin and euglobulin fractions expressed in terms corresponding to the original serum was then found to be:—

	100	200	300	400	500	800	1000
Pseudoglobulin	+++	+++	+++	+++	+++	++	+
Euglobulin	++	-	-	-	-	-	-

Table IV concisely explains the results of this experiment.

TABLE IV.

	Dilutions of serum				
	100	200	500	800	1000
Original Serum	+++	+++	+++	+++	++
Total Globulin	+++	+++	+++	++	+
Pseudoglobulin	+++	+++	+++	++	+
Euglobulin	++	-	-	-	-

+++ indicate large, ++ moderate sized, + small clumps, - a negative reaction.

Does immunising an animal against the Meningococcus increase in its blood the agglutinins for the *B. aquatilis alcaligenes*?

Two rabbits were inoculated with cultures of the Meningococcus at intervals of 10 days for three months. At the end of this time the agglutinins for the Meningococcus were but slightly increased and those for the *B. aquatilis alcaligenes* not at all.

One of the rabbits was now given two inoculations with the

*B. aquatilis alcaligenes* as the result of which its blood serum agglutinated this bacillus in a dilution of 1 in 2000 in  $\frac{1}{2}$  hour but the agglutinins for the Meningococcus were not increased.

The action of various antimeningococcic sera was now tested on the Meningococcus (strain G) and on the *B. aquatilis alcaligenes*.

The results obtained are expressed in Table V.

TABLE V.

Test organisms	Dilutions of serum									
	5	10	20	40	60	80	100	200	300	400
Serum No. I a:										
Meningococcus (Strain G)	+++	+++	+++	+++	+++	+++	++	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	+++	+++	+++	+++	++	+
Serum No. I b:										
Meningococcus (Strain G)	+++	+++	+++	+++	+++	+++	+++	+	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	+++	+++	+++	+++	++	+
Serum No. II:										
Meningococcus (strain G)	-	-	-	-	-	-	-	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+	-	-	-	-	-	-
Serum No. III:										
Meningococcus (Strain G)	-	-	-	-	-	-	-	-	-	-
<i>B. aquatilis alcaligenes</i>	+++	+++	+++	+	-	-	-	-	-	-
Three specimens of normal horse serum:										
1. <i>B. aquatilis alcaligenes</i>	+++	+++	+++	+++	++	+	+	-	-	-
2. " "	+++	+++	+++	+++	+++	++	++	-	-	-
3. " "	+++	+++	+++	+++	++	+	+	-	-	-

These results although not convincing tend to show that when a horse has been immunised against the Meningococcus and has agglutinins for this organism present in its serum that along with them agglutinins for the *B. aquatilis alcaligenes* are produced in excess.

Does immunising an animal against the *B. aquatilis alcaligenes* produce in its blood agglutinins for the Meningococcus?

A rabbit was inoculated subcutaneously on two occasions with two agar cultures of the *B. aquatilis alcaligenes*.

Before the first inoculation the agglutinative action of its blood serum on the Meningococcus and on the *B. aquatilis alcaligenes* was as follows:—

	Dilutions of serum			
	2	5	10	20
<i>B. aquatilis alcaligenes</i>	...	...	-	-
Meningococcus (Strain G)	-	-	-	-

At the end of 20 days the agglutination titre was found to be

	2	5	10	20	500	1000	1500
<i>B. aquatilis alcaligenes</i>	...	...	...	+++	+++	+	-
Meningococcus (Strain G)	-	-	-	-	-	-	-

We conclude then that in the rabbit at any rate inoculation with *B. aquatilis alcaligenes* does not produce in its blood serum agglutinins for the Meningococcus. We may note that the rabbit which has been inoculated with Meningococci and then received two injections of the *B. aquatilis alcaligenes* seemed to form more agglutinins for the *B. aquatilis alcaligenes* than the rabbit which received the same number of injections of the *B. aquatilis alcaligenes* alone.

An interesting and practical question arises here "Does inoculation of an animal with cultures of the Meningococcus and of the *B. aquatilis alcaligenes* lead to the production of a more powerful antimeningococcic serum than that produced when the Meningococcus alone is used?" We can give no answer to this question at present.

#### EXAMINATION OF THE BLOOD SERUM IN CASES OF TYPHUS FEVER.

##### *Agglutinative action on the *B. typhosus*.*

Thirty-one different samples of the blood serum obtained from Belfast cases of typhus fever were examined as to their action on the *B. typhosus*, with the result that 18 gave a positive and 13 a negative reaction when a 1:50 dilution of the serum was used.

On several occasions different strains of typhoid bacilli were employed and almost invariably specimens of blood from non-typhus cases were simultaneously examined with reference to the Widal test, and so these served to control our results.

In two cases there was still a trace of agglutination with a 1:200 dilution. In a few of the cases there was a history of a previous attack of typhoid fever but in the majority there was no such history, and in one of the cases (a young girl) in which the serum gave a positive reaction in a dilution of 1:200, minute enquiry failed to elicit any account of a preceding illness.

##### *Agglutination of a bacillus isolated from the stools of a typhus fever case.*

On smearing a loopful of faeces obtained from a typhus fever case over a Conradi-Drigalski plate red and blue colonies in equal

number developed. On subculture the blue colonies proved to be composed of bacilli having the following characters.

Morphology :—bacilli very similar in appearance to typhoid bacilli but non-motile,—Gram-negative.

Cultural characters :—the growth on agar and gelatin media is similar to that of the *B. typhosus*. On gelatine plates it forms transparent “vine leaf” surface colonies :—no liquefaction of the gelatin results.

Bouillon and peptone water :—uniform turbidity. Well marked indol reaction at the end of four or five days.

Potato :—whitish almost invisible growth.

Litmus milk first becomes slightly acid, then more alkaline, finally becomes acid again and clots. The action of this bacillus on lactose is peculiar. On solid media containing this substance (e.g. that of Conradi-Drigalski) the colonies are blue, there being no evidence of acid production. In lactose litmus broth no change beyond the production of a uniform turbidity is visible until the fifth day when the medium becomes slightly acid, later the acidity becomes more marked and finally gas is also produced.

Glucose, mannite, dulcrite and arabinose are fermented with the production of acid and gas.

Raffinose shows no change.

It is evident from the above description that this organism, which for convenience of reference we shall name the *Bacillus U*, is a variant strain of the *B. coli*.

When first isolated the *Bacillus U* was agglutinated by normal serum in a 1:60 but not in a 1:100 dilution. A sample of typhus serum agglutinated it at this time in a 1:300 dilution. After a few subcultures normal serum agglutinated the bacillus slightly with a 1:300 dilution whilst the effect of typhus serum was proportionately increased.

In Table VI is shown the agglutinative action of the blood serum of seven typhus fever cases (the only ones examined) and of three normal adults on this bacillus.

From a consideration of this Table it is evident that the blood serum of typhus fever cases has on an average five times the agglutinative action of normal serum on the *Bacillus U*.

I may add that these patients were undoubtedly suffering from typhus fever. The clinical picture was typical and in nearly all cases many members of the same family were attacked. Moreover the blood

*Heterologous Agglutinins*

was examined by cultural methods in 32 cases and the flasks either remained sterile or there was a growth of Diplococci but never of bacilli.

We never obtained the *Bacillus U* from any part of the body except the intestine. The fact that the *Bacillus U* was never obtained from the blood would seem to show that it had no etiological connection with the disease.

TABLE VI.

Name	Dilutions of serum employed								
	100	200	300	400	500	600	800	1000	
F. M <sup>o</sup> C., 4 wks. convalescent	+++	+++	+++	+++	+++	+++	++	++	
E. U. , "	+++	+++	+++	+++	+	+	-	-	
H. U. , "	+++	+++	+++	+++	+++	+++	++	++	
S. U. , "	+++	+++	+++	+	-	-	-	-	
N. T. , "	+++	+++	+++	+++	+++	+++	+++	++	
M. C. , "	+++	+++	+++	+++	+++	+++	+++	+++	
B. , "	+++	+++	+++	+++	+++	+++	+++	+++	
R. M. S., normal	+++	+++	+	-	-	-	-	-	
W. J. W. , "	+++	+++	+	-	-	-	-	-	
T. H. , "	+++	+++	+	-	-	-	-	-	

Name	Dilutions of serum employed								
	1200	1400	1600	1800	2000	3000	3500	4000	
F. M <sup>o</sup> C., 4 wks. convalescent	++	+	+	-	-	-	-	-	
E. U. , "	-	-	-	-	-	-	-	-	
H. U. , "	-	-	-	-	-	-	-	-	
S. U. , "	-	-	-	-	-	-	-	-	
N. T. , "	++	+	-	-	-	-	-	-	
M. C. , "	++	+	+	-	-	-	-	-	
B. , "	+++	+++	+++	+++	+++	+++	++	++	
R. M. S., normal	-	-	-	-	-	-	-	-	
W. J. W. , "	-	-	-	-	-	-	-	-	
T. H. , "	-	-	-	-	-	-	-	-	

After we had made the above observations on the action of the blood serum of typhus fever on the *Bacillus typhosus* and on the *Bacillus U* we read with interest a paper by Dr T. Horiuchi (1908).

This observer made a bacteriological study of 40 cases of a fever which occurred amongst the Japanese troops during the Russo-Japanese War. From the faeces of some of the patients he cultivated a bacillus on which the blood serum of the cases had a high agglutinative effect. Culturally his bacillus is identical with the *Bacillus U* described by me. In three cases Horiuchi obtained the same bacillus from the urine but the blood of 40 cases although examined daily gave no growth.

The fever ran a course like typhus but whether it was genuine typhus fever was left an open question. Horiuchi calls the disease "Febris exanthematicus Mandschurici" and his bacillus "Bacillus febris exanthematici Mandschurici." He also found that in some cases the blood serum agglutinated the *B. typhosus* and regarded this as an instance of a group reaction.

The fact that the blood serum of typhus fever cases in Manchuria and in Ireland should have been independently discovered to have an agglutinative action on an intestinal organism is rather interesting, but whether the phenomenon should be taken as an instance of specific or of heterologous agglutination we must leave for the present undecided.

These observations show that the Widal test cannot be relied upon to distinguish typhus from typhoid fever.

Those who have much experience in the use of this test recognise its great value but they also know that occasionally a positive result is obtained with the blood serum of patients whom clinical and pathological evidence eventually proved not to have been infected with the *B. typhosus*.

We shall briefly refer to a few striking instances of this, mentioning the names of the observers, the diagnosis and the dilution of the serum employed in the test.

(1) In cases of infection with staphylococci and streptococci:—

Megelé (1903). A case of liver abscess from the pus of which a staphylococcus aureus was obtained in pure culture.

Heinrich Gräf (1906). A case of streptococcal septicaemia. Dilution of serum 1 in 100.

Lommel (1902). A case of puerperal fever. 1 : 80.

Hale White and Pakes (1902). A case of streptococcal endocarditis. 1 : 200.

(2) Pneumonia:—

Kassel and Mann (1899). Two cases of croupous pneumonia. 1 : 50.

Jul. G. Iversen (1905) gives details of a very interesting case. This was a patient 21 years of age. The agglutination test was six times employed. On the 11th and 13th days of the disease there was no agglutination with a 1 : 50 dilution. On the 16th and 19th days the reaction was positive with 1 : 500 dilution. On the 20th day clumping still occurred with a 1 : 1500 dilution and the same result was obtained with the serum taken at the autopsy. The post-mortem examination

revealed diphtheria bacilli and Streptococci in the throat and Streptococci were also cultivated from the spleen, liver and tonsils. The lungs showed a croupous pneumonia with Fraenkel's Pneumococcus in the exudate. There was no evidence either anatomical or bacteriological in favour of infection with the *B. typhosus*. There was a slight catarrhal enteritis.

(3) In Meningitis:—

Van Oordt (1897). A case of pneumococcal endocarditis and meningitis. 1:40.

Jez (1897) and Marcuse (1908). Cases of tubercular meningitis. 1:100.

Symmers and Wilson (1907). A case of meningococcal meningitis. 1:200.

(4) In Tuberculosis:—

Jul. G. Iversen (1905). Three cases of pulmonary tuberculosis giving positive reactions with dilutions of 1:250, 1:250 and 1:50 respectively.

Ernest Krencker (1908) examined the serum of 26 cases of tuberculosis and found that eight cases gave a positive reaction with a 1:50 dilution and of these eight, three were still positive with a 1:200 dilution.

#### CONCLUSIONS.

1. The results obtained (see *Journ. of Hyg.* 1908, Vol. VIII. No. 3, p. 313) as to the agglutinative action of the blood serum of cerebro-spinal fever cases on the *B. typhosus*, *B. coli* and *B. aquatilis alcaligenes* are confirmed and extended.

2. The general setting of these results with regard to the main facts in the literature of agglutination is undertaken and the conclusion is reached that these secondary agglutinins do not indicate a mixed infection but are of the nature of heterologous "Nebenagglutinine." If this is so the observations of Professor Symmers and myself are of a similar nature to those recorded by Posselt and Sagasser, Hetsch and Lentz, Ballner and Sagasser and Park and Collins.

3. The course of development of the agglutinins for the Meningococcus and for the *B. aquatilis alcaligenes* in the blood of a case of cerebro-spinal fever was followed from day to day. When the results obtained were represented by curves a certain degree of parallelism between the latter was found to exist.

4. The heterologous agglutinins are bound up with the globulin component of the serum: the greater part belongs to the "pseudo-globulin," a smaller part to the "euglobulin" fraction.

5. Heating the serum at 60° C. for 10 minutes destroys completely the agglutinins for the Meningococcus but only partially those for the *B. aquatilis alcaligenes*. Heating at 65° C. for 10 minutes completely destroys the agglutinins both for the Meningococcus and for the *B. aquatilis alcaligenes*.

6. The blood serum of 18 out of 31 cases of typhus fever examined was found to agglutinate the *B. typhosus* in 1 : 50 dilution. The Widal test cannot be relied on to distinguish typhoid from typhus fever. Blood cultural methods are preferable.

7. Seven cases of typhus fever were examined as to their action on a bacillus which had been isolated from the stools of one of the cases. On an average the fever cases had five times the agglutinative action of normal serum.

It gives me great pleasure to acknowledge my indebtedness to Professor Symmers and Dr A. Gardner Robb of Belfast for every facility afforded me to make the observations and experiments recorded in this paper.

Finally I have to thank Professor Rubner for giving me permission to make free use of the volumes in the Library of the Hygienic Institute of Berlin University.

#### REFERENCES.

- BALLNER and SAGASSER (1904). Über spezifische Bindung von Agglutininen bei Absorptionsversuchen. *Archiv f. Hyg.*, LI. 266.
- BORDET, A. (1899). Mécanisme de l'agglutination. *Annales de l'Institut Pasteur*. Footnote p. 248.
- BÜRGI (1907). Über Bakterienagglutination durch normale Sera. *Archiv f. Hyg.*, LXII. 239.
- CASTELLANI, A. (1902). Agglutination bei gemischter Infektion u. Diagnose d. letzteren. *Zeitschr. f. Hyg.*, XL. 1.
- COLLINS, KATHERINE R. (1908). The production of Agglutinins in the animal body by the inoculation of substances other than products of bacterial origin. *Journ. Exper. Med.* x. 529.
- DUNGERN, v. (1903). Spezifität der Antikörperbildung. *Festschrift f. Robert Koch*, p. 1.
- DURHAM (1900). Some theoretical considerations upon the nature of Agglutinins

- together with further observations upon *B. typhi* abdom. etc. *Journ. Exper. Med.*, v. 353.
- ECKARDT (1902). Widalsche Serumreaktion bei Weilscher Krankheit. *München. med. Wochenschr.*, No. 27, p. 1129.
- EISENBERG and VOLK (1902). Untersuchungen über die Agglutination. *Zeitschr. f. Hyg.*, XL. 155.
- FRAENKEL and OTTO (1897). Experimenteller Beitrag zur Lehre von der Agglutinationswirkung des Typhusserum. *München. med. Wochenschr.*, No. 39, p. 1065.
- GIBSON and COLLINS (1907). On the Fractionation of Agglutinins and Antitoxin. *Journ. of Biological Chemistry*, III. 233.
- GRÄF, HEINRICH (1906). Zur bakteriologischen Typhusdiagnose. *Zeitschr. f. Hyg.*, LIV. 205.
- GRUBER and DURHAM (1896). Theorie der aktiven u. passiven Immunität gegen Cholera, Typhus und verwandte Krankheitsprozess. *München. med. Wochenschr.*, No. 13, p. 285.
- GRÜNBAUM (1896). Preliminary note on the use of the agglutinative action of human serum for the diagnosis of Enteric Fever. *Lancet*, II. 806.
- (1896). On the agglutinative action of human serum in its relation to the sero-diagnosis of Enteric Fever. *Lancet*, II. 1747.
- (1897). Über den Gebrauch der agglutinierenden Wirkung von menschlichem Serum für die Diagnose des Abdominaltyphus. *München. med. Wochenschr.*, No. 30, p. 330.
- HETSCH and LENTZ (1903). Beitrag zur Frage nach der Specifität der im Serum des normalen u. Choleraimmunisierten Pferdes enthaltenen Agglutinine. *Festschrift f. Robert Koch*, p. 17.
- HIRSCHFELD (1907). Untersuchungen über die Hämagglutination und ihre physikalischen Grundlagen. *Archiv f. Hyg.*, LXIII. 237.
- HORIUCHI, T. (1908). Über einen neuen Bacillus als Erreger eines exanthematischen Fiebers in der Mandschurei während des japanisch-russischen Krieges, "Bacillus febris exanthematici Mandschurici." *Centralbl. f. Bakteriol.*, XLVI. Originale, p. 586.
- IVERSEN, JUL. G. (1905). Über die Schwankungen des Agglutinationsvermögens des Serums im Verlaufe des Typhus abdominalis. *Zeitschr. f. Hyg.*, XLIX. 1.
- JÄGER (1892). Die Aetiologie des infectiösen fieberhaften Icterus (Weilsche-krankheit). *Zeitschr. f. Hyg.*, XII. 525.
- JATTA, M. (1900). Experimentelle Untersuchungen über die Agglutination des Typhusbacillus und der Mikroorganismen der Coligruppe. *Zeitschr. f. Hyg.*, XXXIII. 185.
- JEZ (1897). Über die Bedeutung der Widal'schen Serodiagnostik. *Wien. med. Wochenschr.*, No. 3.
- JÜRGENS (1903). Beobachtungen über die Widalsche Reaktion und die Mitagglutination von Typhoidbazillen. *Zeitschr. f. Hyg.*, XLIII. 372.
- JÜRGENS, CONRADI and DRIGALSKI (1903). *Zeitschr. f. Hyg.*, XLIII. 141.
- KASSEL and MANN (1899). Lehre von der Gruber-Widalschen Serumdiagnose des Unterleibstypus. *München. med. Wochenschr.*, No. 18.
- KRAUS and LÖW (1899). Über Agglutination. *Wien. klin. Wochenschr.*, No. 5.

- KRENCKER, ERNST (1909). Typhusagglutination bei Tuberkulose. *München. med. Wochenschr.*, LVI. 1016.
- LANDSTEINER and REICH (1908). Über den Immunisierungsprozess. *Zeitschr. f. Hyg.*, LVIII. 213.
- LOMMEL (1902). Eine Fehldiagnose auf Grund der Gruber-Widalschen Reaktion. *München. med. Wochenschr.*, No. 8, p. 314.
- LUBOWSKI and STEINBERG (1904). Über Agglutination der Typhusbazillen bei Proteus u. Staphylokokken Infektion. *Deutsches Archiv f. klin. Med.*, LXIX.
- MARCUSE (1908). *La Semaine médicale*, No. 51, p. 611.
- MAMLOK (1908). Beiträge zur Frage der Normalagglutination. *Archiv f. Hyg.*, LXVIII. 95.
- MEGELÉ (1903). Widalsche Serumreaktion bei Leberabscess. *München. med. Wochenschr.*, No. 14, p. 598.
- MÜLLER, G. (1901). *Über Agglutination*. Dissertation. Berne 1901.
- ÖBERMEYER and PICK (1904). Über den Begriff der Art und Zustandsspezifität und die Beeinflussung der chemischen Eigenart des Tierkörpers. *Wien. klin. Wochenschr.*, x. No. 3.
- ORDT (1897). Zur Serodiagnostik des Typhus abdominalis. *München. med. Wochenschr.*, No. 13.
- PALTAUF, R. (1904). [An invaluable article on Agglutination] in Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, Vol. iv. Part I. p. 668.
- PARK (1908). Pathogenic Microorganisms. London. Henry Kimpton. P. 174. The original article of Park and Collins appeared in the *Journal of Medical Research*.
- PFAUNDLER (1899). Über Gruppenagglutination. *München. med. Wochenschr.*, No. 15, p. 472.
- Zur Serodiagnostik im Kindersalter. *Jahrb. f. Kinderheilk.*, L. 295.
- POSSELT and SAGASSER (1903). Über Beeinflussung der Agglutinine durch spezif. Absorptionen. *Wien. klin. Wochenschr.*, No. 24.
- SCHUMACHER (1901). Uebergang der Agglutinine auf den Fötus. *Zeitschr. f. Hyg.*, XXXVII. 323.
- STEINBERG (1904). Über Agglutination von Typhusbazillen durch das Blutserum. *München. med. Wochenschr.*, No. 11, p. 468.
- STERN (1903). Über den Wert der Agglutination für die Diagnose des Abdominaltyphus. *Berliner klin. Wochenschr.*, Nos. 30 and 31, p. 681 and p. 712.
- SYMMERS, W. ST CLAIR and WILSON, W. JAMES (1907). On the cultivation of the Meningococcus in the present epidemic of cerebro-spinal meningitis in Belfast. *Brit. med. Journ.*, I. 1477.
- (1907). Epidemic cerebro-spinal meningitis with Typhoid Agglutinins in the blood. *Brit. med. Journ.*, II. 713.
- (1908). Agglutination of Bacilli of the alkaligenes, colon and typhoid groups by the blood serum of cases of Cerebro-Spinal Fever. *Journ. of Hyg.*, VIII. 313.
- (1909). Some points bearing on the bacteriology of Cerebro-Spinal Meningitis. *Journ. of Hyg.*, IX. 9.

- VERNEY. Über die gegenseitige Wirkung auf einander folgender Immunisierungen im Tierorganismus. *Centralbl. f. Bakteriol.*, XXXII., 290 and 366.
- WASSERMANN (1903). Über Agglutination und Präcipitation. *Zeitschr. f. Hyg.*, XLII. 267.
- WHITE and PAKES (1902). *London Clinical Soc.*, 12 March.
- WIDAL (1896). Sérodiagnostic de la fièvre typhoid. *Soc. méd. des hôp.*, 26 June.
- WILSON, W. JAMES (1907). The value of the Widal reaction in the diagnosis of Typhoid Fever as ascertained by its use on 230 occasions. *Medical Press and Circular*, 26 June.
- ZUPNIK (1902). Widalsche Serumreaktion bei Weilscher Krankheit. *München. med. Wochenschr.*, No. 31, p. 1305.

A COMPARISON BETWEEN THE GERMICIDAL POWER  
OF A DISINFECTANT IN SOLUTION AND IN THE  
EMULSIFIED STATE.

By R. E. MASSEY.

*Member of the Pharmaceutical Society.*

(*From the Bacteriological Laboratory, King's College, London.*)

(1 Text Figure.)

IN the Milroy Lectures of this year, Professor Hewlett stated<sup>1</sup>:— “There is some evidence to show that the germicidal power of emulsified disinfectants is greater than can be accounted for by the activity of the active constituent and theoretically seems likely, but we want some reliable experimental work in confirmation. It is true that Chick and Martin<sup>2</sup> have compared the germicidal value of higher tar acids emulsified in water and dissolved in alcohol, their results showing that the emulsified form was much more active; but the reliability of this work is vitiated by the fact that they have entirely failed to take into account the influence of alcohol in decreasing germicidal activity; for example in the case of phenol, which I dealt with in my last lecture.”

Rideal and Walker<sup>3</sup> also claim that an emulsion of trikresol has a germicidal power three times greater than a solution of the same concentration, but in this case the comparison was imperfect, a resin soap being used for the emulsion and an oleate for the solution.

The factors determining the accuracy of disinfectant experiments are so delicate that concordant results can only be obtained when the conditions under which the comparison is instituted are precisely similar.

It is obviously impossible to prevent a soluble substance from going into solution; that is, without changing its composition, and thus altering its properties; the most that can be hoped for, is that the rate of solution will be retarded. The range of solvents, also, is limited, since

<sup>1</sup> *Lancet*, 1909, i. p. 893.

<sup>2</sup> *Journ. of Hygiene*, 1908, viii. p. 698.

<sup>3</sup> *Journ. Sanitary Inst.*, 1903, xxiv. p. 425.

many have, in themselves, antiseptic or germicidal action ; and, as the substance will only remain in the state of suspension for a short space of time, it is necessary that the diluent should be inert for the difference to become appreciable.

A weak emulsion of tragacanth, when kept between 20° and 40° C. was found to delay solution of phenol for about 15 minutes, and, in the case of the difficultly soluble cresols, the particles could be detected after 3 hours.

It also has the merit of being perfectly homogeneous, and thus does not depreciate the germicidal power, as particulate matter has been found to do. It is quite inert, and, by using the same sample of tragacanth, an emulsion, similar in properties, can be prepared for each experiment.

Accordingly, 4 gm. of tragacanth (sterilised) was placed in a sterile graduated cylinder, and moistened with 2 c.c. of absolute alcohol ; about 80 c.c. sterile water were now added, and the cylinder well shaken. The mucilage was then sterilised in an autoclave at 120° C. for 10 minutes and allowed to stand over-night, that the liquid might be freed from air bubbles. The volume was finally made up to 100 c.c. less the amount of disinfectant to be added.

This represented the basis for the emulsion. When preparing the solution, the disinfectant was dissolved in the sterile water before being added to the tragacanth, and, after standing some time, the product made up to 100 c.c. This was not sterilised in the autoclave.

After placing 20 c.c. of the tragacanth basis in a sterile Erlenmeyer flask, and withdrawing an amount equal in volume to that of the disinfectant to be used, the measured amount of cresol or phenol was added, and the whole gently rotated. A known quantity of the organism was quickly added and subcultures were made in the Rideal-Walker manner, strict precautions being taken to ensure constancy in time, in quantity added to each subculture (3 mm. loop bent at a right angle to the surface of the liquid), and in temperature.

To 20 c.c. of the solution the same amount of culture was added, and subcultures were made in a manner precisely similar to that used for the emulsion.

The following results were obtained :

Exp. I. Temp. 20° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask, and subcultured every 2 minutes.

- A. 20 c.c. 1 % emulsion of phenol.
- B. 20 c.c. 1 % aqueous solution of phenol (without tragacanth).

*Result* :—No growth in either case after 2 minutes.

Exp. II. Temp. 15·5° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask, and subcultured every 2 minutes.

- A. 20 c.c. 1 % emulsion of phenol kills in 4 minutes.
- B. 20 c.c. 1 % solution of phenol kills in 6 minutes.

Exp. III. Temp. 17° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 60 seconds.

- A. 1 in 120 emulsion of phenol kills in 6 minutes.
- B. 1 in 120 solution     ,     11     ,

Exp. IV. Temp. 20° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 30 seconds.

- A. 1 in 120 emulsion of phenol kills in 4·5 minutes.
- B. 1 in 120 solution     ,     6     ,

Exp. V. Temp. 18° C. 1 c.c. 24 hrs. old Typhoid broth added to each flask and subcultured every 30 seconds.

- A. 0·9 % emulsion of phenol kills in 1 minute.
- B. 1 % solution     ,     ,     1     ,

Exp. VI. Temp. 16° C.

- A. 0·9 % emulsion of phenol kills in 1½ minutes.
- B. 1 % solution     ,     ,     1½     ,

It will be seen from the above that although the temperature and strength of disinfectant were varied, the emulsion was slightly stronger in each case. In order to demonstrate more clearly the difference in strength, a hardier organism (*B. coli*) was subjected to experiment.

Exp. VII. Temp. 20° C. 1 c.c. *B. coli* broth 24 hrs. old added to each flask, and subcultured every 30 seconds.

- A. 1 % emulsion of phenol kills in 2½ minutes.
- B. 1 % solution     ,     ,     4     ,

It has been stated elsewhere that the phenol could be maintained in the emulsified state for a short time only. In order to lessen this difficulty, the more slowly soluble trikresol was used in the remaining experiments.

Exp. VIII. Temp. 18° C. 1 c.c. *B. coli* broth, 24 hrs. old, added to each flask and subcultured every 30 seconds.

- A. 0·4 % emulsion of trikresol kills in 3½ minutes.
- B. 4 % solution     ,     ,     ,     6½     ,

A still more resistant organism is the *Staphylococcus pyogenes aureus*. A 24 hr. culture on slope agar was taken and emulsified with sterile water.

Exp. IX. Temp. 20° C. 1 c.c. suspension of *Staphylococcus* added to each flask, and subcultured on to slope agar at intervals of 30 seconds for 5 minutes.

- A. 5 % emulsion of trikresol.
- B. 5 % solution     ,

*Result*:—The death point was not reached, but the difference in the amount of growth was very marked, for whereas, that exposed to the action of the solution was vigorous, the colonies after treatment with the emulsion were small and scanty in number.

The uniform success attending these experiments suggested that a quantitative result might be obtained. It was recognised that although

the emulsion would not persist until the end of the experiment, yet the ratio obtained during the first half would be maintained throughout. Accordingly a thin emulsion was made of an old sporing culture of *B. mycoides*, and a loopful of this added to the emulsion and solution of the disinfectant. Loopfuls were sowed into agar plates at intervals, and after incubation the colonies were counted.

In the first experiment, the emulsion was too thick and the plates so crowded that the individual colonies could not readily be enumerated. An appreciable difference was noted, and a rough count taken. On dividing the number of colonies from the solution by the number from the emulsion, an interesting ratio was obtained.

Time	Ratio Solution Emulsion
After 1 hour	1.15
2 hours	2.24
3 „	1.68
3½ „	1.21
3¾ „	1.20
4 „	1.18

Temp. 40° C.

The steepness of the curve obtained from the results of the next experiment during the first two hours of action verifies these figures, and seems to indicate that the germicidal power gradually decreased as the trikresol passed into solution, which happened rather quickly in this experiment owing to the high temperature. The suspension of spores was therefore diluted and the experiment repeated, with the following results.

#### *Spores of B. mycoides.*

Temp. 30° C. 1 loopful of suspension added to each flask.

Time	Emulsion 2 % Trikresol	Solution 2 % Trikresol
	No. of colonies	No. of colonies
1 hour	85	98
2 hours	52	72
2½ „	43	66
3 „	36	56
3½ „	32	54
3¾ „	28	48
4½ „	21	37
5½ „	15	31

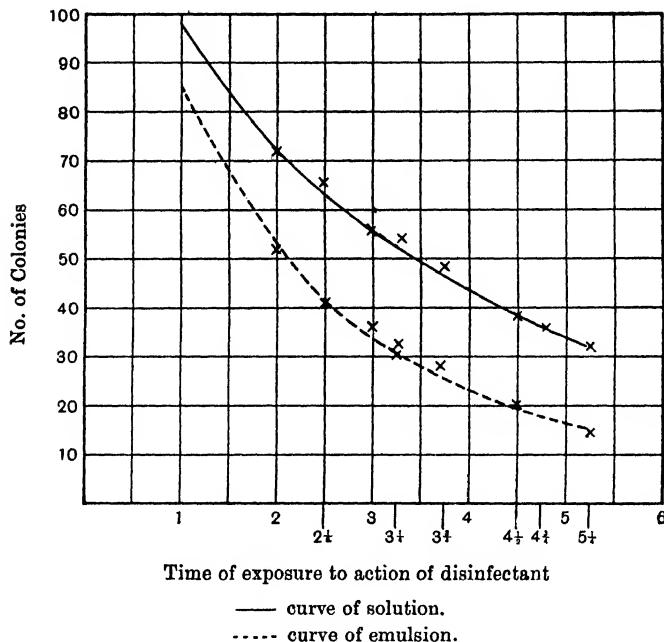
1 loopful 3 mm. diameter sowed into each plate. A graphic representation of these figures renders the difference more marked.

The course of action of a disinfectant on bacterial spores has been shown by Miss Chick<sup>1</sup> to progress according to the formula

$$K = \frac{1}{t_2 - t_1} \log \frac{n_1}{n_2},$$

where  $n_1$  and  $n_2$  are the number of organisms surviving after times  $t_1$  and  $t_2$  respectively.

*Disinfection of Spores of B. mycoides by an emulsion and by a solution of Trikresol. Temp. 30° C.*



When this is applied to the number of colonies given above the following constants are obtained.

$K$ for emulsion	$K$ for solution	Ratio $\frac{K \text{ from emulsion}}{K \text{ from solution}}$
.213	.134	1.59
.197	.114	1.72
.187	.121	1.54
.188	.115	1.6
.175	.113	1.54
.174	.121	1.44
.177	.117	1.51

<sup>1</sup> *Journ. of Hygiene, 1908, viii. p. 94.*

It will be noted that throughout the experiment the constant of the emulsion is higher than that of the solution, but the ratio of the two is nearly the same. Moreover there is a continued decrease in value as the process of disinfection goes on.

The experiments were identical in every respect, and done simultaneously; the larger value of  $K$  therefore indicates that the rate of disinfection is greater in the case of the emulsion. The even ratio may be accounted for by the fact that the actual germicidal action is similar in each case, and also that emulsion was not changing to solution.

The gradual decrease exhibited in both emulsion and solution must be the result of the number of spores being lessened; the survivors may be the more resistant forms.

No exact accuracy is claimed for the above figures, the purpose in view being simply to demonstrate the superior germicidal power of the emulsified trikresol, and not to indicate the exact rate of disinfection. The unequal distribution throughout the basis probably accounts for the lack of that symmetry which characterises the curves depicted by Miss Chick.

When a few drops of the emulsified disinfectant were examined under the microscope with a small quantity of typhoid culture, it was evident that the motility of the bacteria was diminished by the viscosity of the liquid. Clumping of the bacteria around a globule of disinfectant (trikresol) could be seen, and it is presumably owing to the fact that a greater concentration exists around these clumps than throughout the solution that the emulsified form exhibits an accelerated rate of action.

In conclusion, the writer's best thanks are due to Professor Hewlett, for continued help during the course of these experiments.

## MISCELLANEA

### FIFTEENTH INTERNATIONAL CONGRESS ON HYGIENE AND DEMOGRAPHY.

WASHINGTON, D.C., SEPTEMBER 26 TO OCTOBER 1, 1910.

Address for letters: Bureau of the Census,  
Division of Vital Statistics, Washington, D.C., U.S.A.

### COMMITTEE ON ORGANIZATION.

DEPARTMENT OF STATE, WASHINGTON, *July 16, 1909.*

The Committee on Organization of the Fifteenth International Congress on Hygiene and Demography has the honour of presenting herewith certain information concerning arrangements, made and in progress, for the forthcoming Congress.

The Fifteenth International Congress on Hygiene and Demography will be held in Washington, September 26 to October 1, 1910, under the auspices of the Department of State of the United States, in pursuance of the Joint Resolution of Congress, approved February 26, 1907, authorizing the President to invite the International Congress of Hygiene and Demography to meet in Washington in 1910.

Under date of May 8, 1909, this general invitation was confirmed by a circular letter of the Department of State addressed to the American ministers and Ambassadors, instructing them to invite the governments to which they are accredited.

## ORGANIZATION.

The following appointments have been made for the purposes of organizing the Fifteenth International Congress on Hygiene and Demography:

*President:* Dr HENRY P. WALCOTT, President of the State Board of Health of Massachusetts.

*Secretary-General:* Dr JOHN S. FULTON.

## COMMITTEE OF ORGANIZATION.

*Chairman:* Hon. HUNTINGTON WILSON, Assistant Secretary of State, Washington.

## MEMBERS.

ABBOTT, Dr A. C., Professor of Hygiene, University of Pennsylvania, Director Bureau of Health, Philadelphia.

BEYER, Dr HENRY G., Medical Inspector U.S. Navy, Washington.

BICKNELL, Mr ERNEST P., Director National Red Cross Society, Washington.

BIGGS, Dr HERMANN M., Medical Director, Department of Health, New York City.

BILLINGS, Dr JOHN S., U.S. Army (Retired), New York Public Library, New York City.

BOARDMAN, Miss MABEL, National Red Cross Society, Washington.

COMMONS, JOHN R., Professor of Political Economy, University of Wisconsin, Madison.

DEVINE, Mr EDWARD T., Secretary of the Charity Organization Society, New York City.

FAVILL, Dr HENRY B., Professor of Therapeutics, Rush Medical College, Chicago.

FULTON, Dr JOHN S., Professor of State Medicine, University of Maryland, Baltimore.

GALLINGER, Hon. JACOB H., U.S. Senator, Washington.

GLENN, Mr JOHN M., Director Russell Sage Foundation, New York City.

JACOBI, Dr ABRAHAM, Emeritus Professor Diseases of Children, College of Physicians and Surgeons, New York.

KOBER, Dr GEORGE M., Professor of Hygiene, Georgetown University, Washington.

LATHROP, Miss JULIA, Hull House, Chicago.

McCAW, Lt.-Col. WALTER D., U.S. Army, Librarian, Surgeon General's Office, Washington.

MELVIN, Dr A. D., Chief of the Bureau of Animal Industry, Washington.

NORTH, Mr S. N. D., Late Director of the Census Bureau, Washington.

OLCOTT, Hon. JOHN VAN VECHTEN, Member of Congress, Washington.

RAVENEL, Dr MAZYCK P., Professor of Pathology and Bacteriology, University of Wisconsin, Madison.

SIMMONS, Dr GEORGE H., Secretary American Medical Association, Chicago.

SMITH, Dr THEOBALD, Professor of Comparative Pathology, Harvard University, Boston.

- VAUGHAN, Dr VICTOR C., President State Board of Health of Michigan, Ann Arbor.
- WALCOTT, CHARLES D., Director of the Smithsonian Institution, Washington.
- WALD, Miss LILIAN, Nurses' Settlement, Henry Street, New York.
- WELCH, Dr WILLIAM H., President State Board of Health of Maryland, Baltimore.
- WILCOX, WALTER F., Professor of Political Economy, Cornell University, Ithaca, N.Y.
- WILBUR, Dr CRESSY L., Chief of Division of Vital Statistics, Bureau of Census, Washington.
- WYMAN, Dr WALTER, Surgeon-General U.S. Public Health and Marine Hospital Service, Washington.

#### NATIONAL COMMITTEES.

Full information concerning National Committees will not be available for several months. These will be formed with the assistance of the—

#### PERMANENT INTERNATIONAL COMMISSION OF THE CONGRESSES OF HYGIENE AND DEMOGRAPHY.

*President*, Geh. Med.-Rat. Prof. Dr RUBNER, Berlin.

*Vice-President*, Hon. S. N. D. NORTH, Washington.

#### MEMBERS.

##### *I. Hygiene.*

- Argentina .....Dr WILDE.
- Belgium .....Dr HYAC. KUBORN.
- Brazil .....Dr BRUNO CHAVES.
- Cuba .....Dr ARISTIDES AGRAMONTE.
- Denmark .....Dr EMIL HOFF.
- Germany .....Geh. Med.-Rat. Dr LOEFFLER.  
Geh. Ober-Med.-Rat. Dr GAFFKY.  
Ober-Med.-Rat. Dr GRUBER.  
Ober-Reg.-Rat. Dr BUMM.
- Greece.....Dr CONSTANTIN SAVAS.
- Great Britain.....Prof. G. H. F. NUTTALL, Mr ALFRED ROECHLING, Dr THEODORE THOMSON.
- Italy .....Prof. Dr LOUIS PAGLIANI.
- Japan .....Prof. TEIGO MATSUCHITA.
- Mexico .....RAMIREZ DE ARELLANO.
- Holland .....Dr W. P. RUYSCHE, Prof. C. H. H. SPRONCK.
- France .....Prof. A. CALMETTE, Prof. CHANTEMESSE, Prof. GEORGES BECHMANN.
- Norway .....Prof. AXEL JOHANNESSEN.
- Austria .....Prof. Dr HUEPPE.
- Russia.....Dr JOSEF POLAK, Dr von RAPCHEWSKI.
- Sweden .....Prof. ERNST ALMQUIST.
- Switzerland .....Prof. FR. ERISMANN.
- Spain .....Dr CORTEZO Y PRIETO.
- Hungary .....Dr von LIEBERMANN.
- United States.....Lieut. Col. WALTER D. McCRAW, Prof. WM. H. WELCH.

*II. Demography.*

- Belgium ..... M. MAURICE SAUVEUR.  
 Austria ..... VON INAMA-STERNEGG.  
 Great Britain ..... DR ARTHUR NEWSHOLME.  
 France ..... DR EMILE LEVASSEUR.  
 Germany ..... DR GEORG VON MAYR.  
 Italy ..... DR LUIGI BODIO.  
 Norway ..... A. N. KIAER.  
 Russia ..... TROITNITSKY.  
 Switzerland ..... DR GUILLAUME.  
 Hungary ..... DR GUSTAV THIRRING.  
 "Membres de droit" ..... MARTIN DUNAUS, DE BEAUFORT, COLOMAN MÜLLER,  
                           C. CALLEJA, GIMENO, E. BECO, F. BUZEYS, HEINRICH  
                           PRINZ ZU SCHONAICH-CAROLATH, I. NIETNER.  
 "Membres Adjoints" ..... VAN ERMENGEM, DEPAIRE, DOCTEUR, RAEMECKERS, Baron  
                           WAHIS.

## REGULATIONS.

## ARTICLE I.

The Fifteenth International Congress on Hygiene and Demography will meet in Washington from the 26th of September to the 1st of October, 1910

## ARTICLE II.

The purpose of the Congress is to promote the knowledge and practice of hygiene and demography.

## ARTICLE III.

Any person, engaged in the study or practice of hygiene or demography, may become a member of the Congress. But the Committee of Organization reserves the right to withhold the privileges of membership in particular cases.

## ARTICLE IV.

The fee for membership is \$5.00 (20 marks=25 francs).

Relatives of members of the Congress, as well as students of colleges and universities, who are not eligible for membership, may become associate members, and may have the privileges of attending the meetings of the Congress, and participating in the entertainments, excursions, and other events occurring in connection with the Congress; but may not vote or participate in the discussions.

These associate members shall pay a fee of \$2.50 (10 marks).

## ARTICLE V.

Each member will receive a report of the transactions of the Congress, and of the protocol, to be published after the adjournment of the Congress.

## ARTICLE VI.

The Congress is divided into ten sections and two divisions.

## I.

- Section 1. Hygienic Microbiology and Parasitology.
- Section 2. Dietetic Hygiene. Hygienic Physiology.
- Section 3. Hygiene of Infancy and Childhood : School Hygiene.
- Section 4. Industrial and Occupational Hygiene.
- Section 5. Control of Infectious Diseases.
- Section 6. State and Municipal Hygiene.
- Section 7. Hygiene of Traffic and Transportation.
- Section 8. Tropical Military and Naval Hygiene.
- Section 9. Hygiene of Institutions and Public Buildings.

## II.

- Section 10. Demography.

**ARTICLE VII.**

The sessions are divided into general sessions and Section sessions. The opening and closing meetings are general sessions. Joint meetings may be arranged between two Sections.

The official languages of the Congress are German, French, and English.

**ARTICLE VIII.**

The Congress is governed by a President, two Vice-Presidents, and Secretaries.

Each Section is governed by a President, Vice-Presidents, and Secretaries.

During the general session for opening the Congress the reports of the Committee on Organization will be acted upon, and honorary presidents of the Congress elected.

During the first session of each Section, the nominations by the President of the Section will be received, and honorary presidents of the Section will be elected.

The President of each Section shall be responsible for the government of his Section throughout the meeting of the Congress ; but the President of a Section may, at his own discretion, relinquish the chair, temporarily, to any of the honorary presidents of the Section.

**ARTICLE IX.**

The Committee on Organization will receive from the Presidents of Sections suggestions as to themes for discussions, and the names of referees and co-referees, and will arrange the program for each of the Sections. The discussion of each theme will be opened and closed by the referee and co-referees chosen for that purpose.

**ARTICLE X.**

Each referee or co-referee must send a short summary or abstract of his communication to the Secretary-General of the Congress before April 1, 1910, so that the abstracts may be sent to the members of the Congress in advance of the meeting. Each referee or co-referee has a maximum time allowance of twenty minutes for the presentation of his paper. After the stated papers are read, the subject will be open for general discussion.

The papers will be arranged in the order in which their notifications are received at the General Office.

During the discussions, each speaker is allowed five minutes, and no speaker may

exceed five minutes without express leave of the Section. No person may speak more than twice on one subject, without the express leave of the Section.

After the general discussion each referee and co-referee is allowed five minutes for closing remarks.

#### **ARTICLE XI.**

Notice of the desire to present a paper or a demonstration must be sent to the President of the appropriate Section, enclosing an abstract or brief account of his proposition. The Presidents of Sections will dispose of these requests in the order of their receipt, and in such a way as not to interfere with the discussion of the official themes.

#### **ARTICLE XII.**

Each speaker, who desires his remarks to be published in the transactions, must hand to the Secretary of the Section, at the close of the session, a typewritten manuscript of his part in the discussion.

Each Section Secretary will make a written report of each session, will take possession of the papers read and the records of discussions, and will hand to the President of the Section an orderly account of the Section proceedings, to be approved by the Presidents and transmitted to the Secretary-General for publication in the transactions.

#### **ARTICLE XIII.**

The Congress will not pass resolutions on scientific questions, but the Sections have the right to offer formal propositions, which may be acted upon by the Congress at the closing session, provided the Permanent International Commission of the Congress of Hygiene and Demography makes no objection.

#### **ARTICLE XIV.**

While the Congress is in session a printed journal will appear daily, giving the program as arranged by the Presidents of Sections, and other information of interest to members of the Congress.

#### **ARTICLE XV.**

At the closing session the report of the Permanent International Commission is received, and the time and place of the next meeting of the Congress is decided.

During the closing sessions, the proposals offered by the Sections are acted upon.

### **DIVISION INTO SECTIONS.**

#### **DIVISION I. HYGIENE.**

##### **SECTION I. HYGIENIC MICROBIOLOGY AND PARASITOLOGY.**

*President:* THEOBALD SMITH, Professor of Comparative Pathology,  
Harvard University, Boston.

##### **SECTION II. DIETETIC HYGIENE. HYGIENIC PHYSIOLOGY.**

*President:* RUSSELL H. CHITTENDEN, Professor of Physiology,  
Yale University, New Haven.

##### **SECTION III. HYGIENE OF INFANCY AND CHILDHOOD : SCHOOL HYGIENE.**

*President:* ABRAHAM JACOBI, Emeritus Professor of Diseases of Children,  
College of Physicians and Surgeons, New York.

## SECTION IV. INDUSTRIAL AND OCCUPATIONAL HYGIENE.

*President:* GEORGE M. KOBER, Professor of Hygiene,  
George Washington University.

## SECTION V. CONTROL OF INFECTIOUS DISEASES.

*President:* HERMAN M. BIGGS, Medical Director, Department of Health  
New York City.

## SECTION VI. STATE AND MUNICIPAL HYGIENE.

*President:* FRANK F. WESBROOK, Professor of Pathology and Bacteriology,  
University of Minnesota, Director of the Laboratory of Hygiene, State Board  
of Health of Minnesota

## SECTION VII. HYGIENE OF TRAFFIC AND TRANSPORTATION.

*President:* WALTER WYMAN, Surgeon-General U.S. Public Health and  
Marine Hospital Service, Washington.

## SECTION VIII. TROPICAL, MILITARY, AND NAVAL HYGIENE.

*President:* HENRY G. BEYER, Medical Inspector U.S. Navy, Washington.

## SECTION IX. HYGIENE OF INSTITUTIONS AND PUBLIC BUILDINGS.

*President:* FRANK BILLINGS, Professor of Medicine, University of Chicago.

## DIVISION II. DEMOGRAPHY.

## SECTION X. DEMOGRAPHY.

*President:* WALTER F. WILLCOX, Professor of Political Economy,  
Cornell University, Ithaca, New York.

BRITISH NATIONAL COMMITTEE FOR INTERNATIONAL  
CONGRESSES FOR HYGIENE AND DEMOGRAPHY.

## OFFICERS.

*President:* Sir SHIRLEY F. MURPHY, M.R.C.S.

*Hon. Secretaries:* {Professor G. H. F. NUTTALL, M.D., F.R.S.  
(H. TIMBRELL BULSTRODE, M.D.)

*Hon. Treasurer:* W. H. HAMER, M.D.

## COMMITTEE.

\*BRANFOOT, Surgeon-General A. M., C.I.E., M.B., F.R.C.S., I.M.S. India Office,  
Whitehall, London, S.W.

BRUCE, Colonel Sir DAVID, C.B., F.R.S., A.M.S., Member of the Army Advisory  
Board, etc. Royal Army Medical College, Millbank, London, S.W.

BUCHANAN, G. S., M.D., B.Sc., Medical Inspector and Chief Inspector of Foods,  
H.M. Local Government Board. Local Government Board, Whitehall, Lon-  
don, S.W.

\* Member of the Council.

- †BULSTRODE, H. TIMBRELL, M.D., Medical Inspector, H.M. Local Government Board; Lecturer in Preventive Medicine, Charing Cross Hospital Medical School. Local Government Board, Whitehall, London, S.W.
- CANTLIE, J., M.B., F.R.C.S., Editor of the *Journal of Tropical Medicine and Hygiene*. 140, Harley Street, London, W.
- \*CHALMERS, A. K., M.D., Medical Officer of Health of the City of Glasgow. Sanitary Chambers, Glasgow.
- CLARKE, Sir ERNEST. 31, Tavistock Square, London, W.C.
- COLES, W. R. E., Secretary of the Plumbers' Company. 1, Adelaide Buildings, London Bridge, London, E.C.
- COLLINGRIDGE, W., M.D., Medical Officer of Health, City of London. Guildhall, London, E.C.
- COWAN, P. C., B.Sc., M.Inst.C.E., Chief Engineering Inspector, Local Government Board, Ireland. 33, Ailesbury Road, Dublin.
- CUTLER, THOMAS W., Fellow of the Royal Institute of British Architects. 5, Queen Square, Bloomsbury, London, W.C.
- DAVIES, Lt.-Col. A. M., M.R.C.S., R.A.M.C., Professor of Hygiene, Royal Army Medical College, Millbank, London, S.W.
- DEAN, GEORGE, M.B., C.M., Professor of Pathology, University of Aberdeen.
- \*DELÉPINE, S., M.B., M.Sc., Professor of Comparative Pathology and Bacteriology, Director of the Public Health Laboratory, York Place, Manchester.
- FIRTH, Lt.-Col. R. H., F.R.C.S., R.A.M.C., Officer in Charge of the School of Sanitation, Aldershot. Baydale, 8, Church Circle, S. Farnborough, Hants.
- GLAISTER, JOHN, M.D., Regius Professor of Forensic Medicine and Public Health in the University of Glasgow. 3, Newton Place, West Glasgow, Scotland.
- GRAHAM-SMITH, G. S., M.D., M.R.C.S., L.R.C.P., University Lecturer in Hygiene. Medical Schools, Downing Street, Cambridge.
- GRÜNBAUM, A. S. F., M.D., F.R.C.P., Professor of Pathology, The University, Leeds.
- \*HALDANE, J. S., M.D., F.R.S., Reader in Physiology in the University of Oxford. Cherwell, Oxford.
- †HAMER, W. H., M.D., F.R.C.P., Medical Officer (General Purposes), London County Council. 55, Dartmouth Park Hill, London, N.W.
- HAY, MATTHEW, M.D., Medical Officer of Health of Aberdeen. Crown Mansions, 42½, Union Street, Aberdeen, Scotland.
- \*HOPE, E. W., M.D., Medical Officer of Health, City and Port of Liverpool. Municipal Offices, Liverpool.
- HORROCKS, Major W. H., M.B., B.Sc., R.A.M.C. Editor of the *Journal of the Royal Army Medical Corps*. Birch Mead, Walton-on-Thames.
- KENWOOD, H. R., M.B., L.R.C.P., Professor of Hygiene and Public Health, University College, Gower Street, London, W.C.
- LEGGE, T. M., M.D., H.M. Medical Inspector of Factories. Home Office, London, S.W.
- LEISHMAN, Lt.-Col. Sir W. B., R.A.M.C., Professor of Pathology, Royal Army Medical College, Millbank, London, S.W.
- LEITH, R. F. C., M.B., F.R.C.P., Professor of Pathology and Bacteriology, The University, Birmingham.

\* Members of the Council.

† Ex-officio members of the Council.

- \*MACKENZIE, W. LESLIE, M.D., M.R.C.P., Medical Member of the Local Government Board. Local Government Board, Edinburgh.
- \*MACPHERSON, Lt.-Col. W. G., C.M.G., M.B., R.A.M.C. War Office, Whitehall, London, S.W.
- \*MANSON, Sir PATRICK, M.D., K.C.M.G., F.R.C.P., F.R.S., Physician to the Colonial Office, etc. 21, Queen Anne Street, Cavendish Square, London, W.
- \*MARTIN, C. J., M.B., D.Sc., F.R.S., Director of the Lister Institute of Preventive Medicine, Chelsea Gardens, London, S.W.
- \*MAY, Deputy Inspector-General ARTHUR W. Medical Department of the Navy, Admiralty.
- MCWEENEY, E. J., M.D., F.R.C.P.I., Professor of Pathology in the Royal University. 84, St Stephen's Green, Dublin.
- MELVILLE, Lt.-Col. CHARLES H., M.B., R.A.M.C. War Office, Whitehall, London, S.W.
- \*MOLINE, PAUL F., M.B., M.R.C.S., Assistant Demonstrator in Practical Physiology, University College, London. 42, Walton Street, Pont Street, Chelsea, London, S.W.
- +MURPHY, Sir SHIRLEY F., Medical Officer of Health of the Administrative County of London. 9, Bentinck Terrace, Regent's Park, London, N.W.
- \*NEWMAN, GEORGE, M.D., Chief Medical Officer of the Board of Education, Whitehall, London, S.W.
- \*NEWSHOLME, ARTHUR, M.D., F.R.C.P., Medical Officer of the Local Government Board, Whitehall, London, S.W.
- NIVEN, JAMES, M.B., B.C., Medical Officer of Health of the City of Manchester. Public Health Office, Town Hall, Manchester.
- NOTTER, J. LANE, Col. (retired), M.D., Army Medical Service. 3, Kensington Mansions, Earl's Court, London, S.W.
- +NUTTALL, G. H. F., M.D., Ph.D., Sc.D., F.R.S., Quick Professor of Biology in the University of Cambridge, Editor of the Journal of Hygiene. 3, Cranmer Road, Cambridge.
- OGLE, WILLIAM, M.D., F.R.C.P. 10, Gordon Square, London, W.C.
- OLIVER, THOMAS, M.D., F.R.C.P., LL.D., Professor of Physiology, University of Durham. 7, Ellison Place, Newcastle-on-Tyne.
- \*PARKES, LOUIS C., M.D., Lecturer on Public Health, St George's Hospital, London; Consulting Sanitary Adviser to H.M.'s Board of Works. 61, Cadogan Square, London, S.W.
- \*ROBERTSON, JOHN, M.D., B.Sc., Medical Officer of Health of the City of Birmingham. Health Department, The Council House, Birmingham.
- \*ROECHLING, H. ALFRED, Consulting and Civil Engineer. 39, Victoria Street, Westminster, London, S.W.
- SANDWITH, F. M., M.D., F.R.C.P., Lecturer at the London School of Tropical Medicine. 31, Cavendish Square, London, W.
- SHELLY, C. E., M.D., M.R.C.P., Fore Street, Hertford, Herts.
- SIMPSON, W. J. R., M.D., F.R.C.P., Professor of Hygiene, King's College, London. 14, Gloucester Place, Hyde Park, London, W.
- SLATER, JOHN, Fellow of the Royal Institute of British Architects. 46, Berners Street, London, W.

\*Members of the Council.

†Ex-officio members of the Council.

- STAFFORD, T. J., C.B., F.R.C.S.I., etc., Medical Commissioner of the Local Government Board of Ireland. Local Government Board, Custom House, Dublin.
- SYKES, J. F. J., M.D., D.Sc., Lecturer in Public Health, Guy's Hospital; Medical Officer of Health of St Pancras. 40, Camden Square, London, N.W.
- TATHAM, JOHN F. W., M.D., F.R.C.P., Superintendent of Statistics. General Register Office, Somerset House, London, W.C.
- \*THOMSON, THEODORE, M.D., C.M.G., Medical Inspector, Local Government Board. Local Government Board, Whitehall, London, S.W.
- \*WALLIS, E. WHITE, Secretary, Royal Sanitary Institute. 74a, Margaret Street, London, W.
- \*WHITELEGGE, B. A., C.B., M.D., F.R.C.P., H.M. Chief Inspector of Factories and Workshops. Home Office, London, S.W.
- WILLIAMS, DAWSON, M.D., F.R.C.P., Editor of the British Medical Journal. 429, Strand, London, W.C.
- \*WOODHEAD, G. SIMS, M.D., Professor of Pathology in the University of Cambridge. The Museums, Cambridge.
- WRIGHT, Sir A. E., M.D., Pathologist to St Mary's Hospital, London. 6, Park Crescent, Regent's Park, London, W.

\* Members of the Council.

#### UNITED STATES ARMY MEDICAL CORPS EXAMINATIONS AT WASHINGTON, CHICAGO AND SAN FRANCISCO.

The Surgeon General of the Army announces that the War Department has appointed permanent boards for the preliminary examination of applicants for appointment in the Medical Corps of the Army to meet at Washington, D.C., Fort Sheridan (near Chicago), Illinois, and San Francisco, California, in addition to the usual preliminary examination boards that are assembled at various Army posts throughout the United States from time to time. The permanent boards will hold sessions on the second Monday of each month.

A limited number of successful candidates will be appointed First Lieutenants in the Medical Reserve Corps (salary \$2,000 per annum) and assigned to Army posts until the next session of the Army Medical School, when they will be ordered to attend the School as "student candidates."

Applicants must be citizens of the United States, between twenty-two and thirty years of age, graduates of reputable medical schools, of good moral character and habits, and shall have had a year's hospital training after graduation, or its equivalent.

Full information concerning the examination can be procured upon application to the "Surgeon General, U.S. Army, Washington, D."

## BRITISH INDUSTRIAL ANTHRAX.

## PART II.

By CECIL H. W. PAGE, M.A., M.D.

## BACTERIOLOGY.

THE number of times that the presence of anthrax bacilli or spores has been detected in imported material is not large. Andrewes (1899) of St Bartholomew's Hospital found anthrax spores in dust shaken from hair taken at random from a suspected bale of Chinese mane hair, hydraulically packed and weighing 5 cwt. Dust taken from the centre of the bale was found infected even after it had been passed, while still hydraulically packed, through a Washington Lyons Steam disinfecter.

Andrew (1900) found anthrax bacilli in a sample of Siberian tail hair, which came from a horsehair factory in Suffolk, and had given rise to a case of anthrax in Glasgow. The hair was undyed, but had been washed in cold water and cleansed by passage through a warm solution of soda and by hackling and drawing into lengths.

Webb and Duncan (1904) of Worcester (County Council Report) found anthrax bacilli in Chinese mane hair, in Russian hair and in Persian wool. Out of eight cases of China hair examined four were found infected. They also found anthrax bacilli in two samples of dust taken from large quantities of hair.

Balfour Stewart (1901) isolated anthrax bacilli from a Kurachee hide and from the dust from a Bombay hide.

MacFadyean (1903) also isolated anthrax bacilli from suspected oats, by making an infusion of the latter in sterile water, and inoculating one sheep and eight guinea-pigs. The sheep and three of the guinea-pigs died. He also isolated anthrax bacilli from suspected cakes.

Anthrax bacilli were isolated from the mud of a stream in Suffolk, into which the waste from a horsehair factory was discharged<sup>1</sup>. A mouse and a guinea-pig after inoculation with the muddy water both died of typical anthrax. Animals grazing on the pastures watered by this stream had died from anthrax.

During the past three years Dr Eurich (1908) and his assistant, Mr Walter Willey have examined for anthrax nearly 600 samples of wool, hair, and dust—not blood-stained—with negative results, while 139 blood-stained specimens have been tested with the result that anthrax bacilli (often in great numbers) were found in 14·4 per cent.

Dr Eurich therefore pointed out that blood-stained material and the dust arising therefrom, with its scales of dried blood, are the means of spreading anthrax spores, and can be called "carriers" of anthrax. A close examination of eight bales of Van-mohair showed that wool etc., which past experience had shown to be especially dangerous, contained a larger percentage of blood-stained fleeces than the others.

Dust may prove dangerous in virtue of the brittle scales of dried blood clots derived from such tainted material.

Klein (1901) in examining samples of "China" tails and Russian manes soaked several grams of the material in steril<sup>e</sup> salt solution, which was then centrifuged and the sediment injected subcutaneously into guinea-pigs and mice. No indication of anthrax was found, but several of the animals died from malignant oedema.

Duncan's method of procedure was apparently similar to that of Klein's, but he found that the bacillus of malignant oedema was often present, and acting more rapidly than anthrax tended to mask it. In cases in which death was due to malignant oedema he removed the spleen, as soon after death as possible, and made cultivations from the central spleen pulp; if anthrax-like colonies developed, a second guinea-pig was infected from these. By these means Duncan says on more than one occasion he has found anthrax, although everything pointed to death from malignant oedema.

In my own research work, putting on one side the methods of isolating the anthrax bacilli by inoculating animals, a series of experiments was first made to determine the best method of isolating the bacillus. About 10 c.c. of sterile horse's serum was placed in a test tube, and infected from a pure culture derived from a cow that had died of anthrax. A few grams of Chinese bristles were then placed in this serum and the whole incubated at 37° C. for 24 hours, after

<sup>1</sup> *Ann. Report of Chief Inspector of Factories*, 1906, p. 291.

which the bristles were removed, and allowed to dry in a Petri dish. Another lot of bristles were similarly infected, using, instead of the horse's serum, sterile salt solution.

From both these artificially infected samples of bristles anthrax bacilli were isolated at various intervals of time. A small quantity of the bristles, about 10 to 20 hairs only, was soaked in sterile salt solution, heated to 40—50° C. and frequently shaken during half an hour. Agar plates were sown directly from this salt solution, using one loopful of the solution to each plate and making three strokes across the plate. After incubating for about 18 hours at 37° C. the plates showed a varying amount of growth; sometimes a more or less thick film spread rapidly over the plate obscuring any other colonies present, others (and the majority) showed a variety of colonies including more or less film.

Agar slopes were found to be of little use, the growth being usually too thick for distinguishing between the various colonies. On nearly all the plates thus made were colonies so like anthrax that it was always difficult to distinguish between them until sub-cultivations were made.

Many experiments were carried out in the hope of eliminating these bacilli without destroying the anthrax bacilli. Bristles were soaked in sterile salt solution for periods varying from half to 24 hours at temperatures from 45 to 60° C. with frequent agitation. These were centrifuged, and the sediment drawn into capillary tubes, and heated at temperatures varying from 80 to 99° C. in water baths. The capillary tubes were again centrifuged and plates made from the sediment.

Control experiments were done at the same time by treating old anthrax spores in a similar manner. In the end it was found impossible to eliminate either the anthrax-like bacilli or those that grew as a film over the plates, obscuring and checking by the rapidity of their growth the development of other bacilli, without at the same time eliminating the small quantity of anthrax likely to be present in any sample of bristles or horsehair.

In a similar manner experiments were made to eliminate these bacilli by incubating bristle samples in carbolic broth of a strength varying from 1 in 1000 to 1 in 1500, but without success.

The method finally adopted in each case was simply to soak a small quantity (1 or 2 grams of the sample) in sterile salt solution. This was warmed to 50° C. by means of a water bath, and was kept at about that temperature for roughly half an hour. Throughout this time the salt solution was frequently shaken. Agar plates were sown directly—one loopful to each plate as above described. In all 83 samples were in-

vestigated as follows ; 44 samples of bristles and 10 of bristle dust were examined and 22 samples of horsehair and 7 of dust. The samples consisted of Chinese (several bundles of the dirty Tientsin riflings being examined), Russian, Siberian, German-Polish, Polish, Indian, French and German bristles. The horsehair consisted of Chinese manes and tails, Siberian manes and tails, Russian tails, South American tails, and mixed Chinese and Russian mane and tail horsehair and cow tails.

Spores of anthrax were not detected in any sample.

Considering that none of the samples consisted of suspected or blood-stained material, and the small quantity actually examined, this result was perhaps to be expected ; at the same time it must be admitted that the method of examination did not with absolute certainty exclude the presence of anthrax, since in several instances plates were spoilt by film-like growths of bacteria rapidly spreading over the agar, and obscuring any other colonies present, and in the second place the presence in relatively large quantities of several different kinds of bacilli very closely resembling anthrax especially in their original colonies upon agar. All those colonies that were likely to be confused with anthrax were subcultured until proved definitely to be of other kinds, these bacilli in pure culture differing in certain respects from anthrax ; and, having proved that anthrax spores can be detected by the above method of plate cultivation, it is a fair inference that anthrax was either not present in any of the samples, or, if present, in infinitesimal quantities.

From bristles and horsehair three types of anthrax-like bacilli were isolated which have been called A, A<sup>1</sup>, and A<sup>2</sup>.

Two examples of type A were isolated, both from Tientsin riflings, differing only slightly from each other in the rate of liquefaction of gelatine, and the readiness with which chains are formed.

Two examples of type A<sup>1</sup> were isolated, one from Indian bristles, and the other from German-Polish bristles.

Of type A<sup>2</sup> several examples were isolated from several different sources.

The characters of these organisms are given in Table I.

An endeavour was made to establish the identity of these three types of bacilli with that of other described bacteria. (See Appendix.)

In drawing comparisons between the anthrax bacillus and the above types of anthrax-like bacilli, it must be remembered that under certain circumstances anthrax does not spike in gelatine stabs, only regaining the property after growing the bacillus on blood agar at 37° C. for

TABLE I.

Type	Name	Description	Original colony on Agar	Gelatine Plate		Remarks
				Surface	Deep	
A	Same size as Anthrax, ends rather rounded,	X X X	Rounded, darker centre, white surrounded with thin chains.	Luxuriant moist irreg, fluffy edge, chains in upper drier parts.	Irrg, dark masses at centre from which numerous spikes extend.	Potato thin and slimy.
A <sup>1</sup>	Same size as Anthrax, with rounded ends, forms chains & filaments broken with difficulty.	X ? ?	Round, white, flat with raised centre, edge shows fine chains.	Rounded at intervals, frills of chains run out.	Curved and wavy edge like Anthrax, surface not so curly.	Gelatine Stab Feathery spikes more numerous near surface, slow surface liquefaction.
A <sup>2</sup>	Same size as Anthrax, corners rounded, stains evenly.	X X X	Round, greyish, from edges fibres pass out, curl, thin, peculiarly like Anthrax.	Moist, slightly granular with fluffy edges.	Small granules.	Surface growth, little deep, very slight liquefaction.
Type	Milk	Gelatine Slope	Agar Plate	Gelatine Plate	Broth	Heat to 80° C.
A	Slightly acid after 5 days, no clot.	X O O O O O	Dulcite Gelatine Slope	At first irregularly rounded with more opaque centre and folded edge, later bundles of irreg. wavy chains.	Slight cloud, no pellicle deposit, sticky white.	Pellicle
A <sup>1</sup>	O	X O O O X X O	Dry granular, very little liq.	Round, numerous chains from edges.	Clear, tangled mass of chains at bottom.	O ? O
Type	Gas	Sacharose Nitrate	Mannite	Mannite		
A <sup>2</sup>	Ac. and cong.	X O O X X X O	Rapid liq., pellicle forms and is wrinkled.	Rounded cols., with numerous thin chains; these often form a network between the cols.	Cloudy, fairly well marked pellicle, stringy white deposit.	O X

24 hours; and further, the anthrax bacillus may frequently show rounded ends.

The A<sup>1</sup> type in many ways is the most like anthrax, and as far as could be determined certainly does not correspond to any known bacillus; though in several cases the descriptions of these non-pathogenic bacilli are meagre in the extreme. The A type also cannot be identified with any known bacillus though it closely resembles one or two, notably 15<sup>o</sup><sup>1</sup>. There can be hardly any doubt that the A<sup>2</sup> type corresponds with the "*Bacillus anthracoides*" of Bainbridge<sup>2</sup>, and it is quite possible it may be a variety of the *Bac. subtilis*. Mr Duncan considered an anthrax-like bacillus, which he isolated from horsehair, to be the *Bacillus subtilis*, and another anthrax-like bacillus, which he also isolated from horsehair, to be the *Bacillus mesentericus vulgaris* of Flügge. Neither the A nor A<sup>1</sup> type can however be said to closely resemble the last-named bacillus.

#### DISINFECTION.

The problem of the destruction of anthrax spores in raw materials without damage to these has still to be solved. It is necessary to find a disinfectant that will overcome the resistance of anthrax spores without injuring the material in any way; further, the price of such disinfectant must be low so as not, by increasing the cost of production, to diminish the power of competing with foreign firms.

The number of means of disinfecting anthrax germs is small; Koch mentions six substances capable of destroying spores of anthrax within 24 hours.

These are, chlorine, bromine, iodine, osmic acid, potassium permanganate, and perchloride of mercury.

The first five are useless, because they are either too expensive or must be used of such a strength (as 5 per cent. potassium permanganate, 3·3 per cent. chlorine or 1 in 10 bleaching powder) as to be damaging to the materials.

Recent experiments show that perchloride of mercury is extremely untrustworthy as it unites with albuminous matter to produce an insoluble compound devoid of germicidal powers. Further, it is extremely poisonous, and has a corrosive action on metals.

<sup>1</sup> Report Local Government Board, 1897-8, pp. 290-295.

<sup>2</sup> Journ. of Bact. and Path. Vol. viii. p. 117, 1903.

Esmarch has shown that anthrax spores have retained their vitality after an exposure of 40 days to five per cent. carbolic, and later experiments confirm this result.

In experiments carried out in the Imperial Health Office at Berlin, turpentine oil was tried, but failed to satisfy the requirements. Formalin vapour also was found unsatisfactory, as for want of penetration it failed to destroy spores placed artificially on bristles.

Eurich of Bradford found a 1% solution of formaldehyde an efficient disinfectant of bales of wool, opened in the bath; and this efficiency was not lost if the bath was used three times. In bales which had been steeped unopened disinfection was found imperfect. More recently he found that formic aldehyde (2½%) subject to the absence of ammonia disinfected bales of wool after two hours steeping. Klein (1901) found that a solution of formalin, 1 in 15, killed anthrax spores in one hour, but failed in three quarters of an hour. The time taken to kill anthrax spores varied with the material to be disinfected.

In the German Health Office potassium permanganate above 2% was found to damage the material, a strength which failed to destroy the spores; but if a 2% solution was used warm, or boiling, for not more than fifteen minutes, with subsequent bleaching with 3—4% sulphurous acid, spores were destroyed.

Sulphurous acid alone must be 11% with a contact of 1—2 hours to be effective, or associated with moisture 5% will kill in 24 hours.

In the products of the distillation of coal besides carbolic acid many bodies of a similar chemical constitution occur, and many mixtures of these are on the market, such as cyllin, izal, lysol, etc. Izal in 10% solution kills virulent anthrax spores in ten minutes (Klein). Commercial cyllin is stated to have a Rideal-Walker co-efficient of 15, it has the advantage of being cheaper than most other disinfectants, and is non-poisonous; while albuminous and other bodies do not affect its efficiency. It is harmless to the skin, metals, wood, and is compatible with soap. Klein found that, taking 45 minutes as the time of exposure, cyllin, diluted 1 in 100 water, was equal to a solution of formalin in water of a strength of 1 in 15; and that cyllin is six times as powerful as formalin in destroying anthrax spores. The spores in this experiment were obtained from a fatal case of wool sorters' disease. Further, Klein found that 1 in 100 cyllin, with a contact of 1½ hours, disinfected efficiently samples of "China" tail and Russian mane horsehair that contained bacilli of malignant oedema, but no anthrax.

For the purpose of the experiment this was satisfactory, as malignant oedema and anthrax are equally resistant.

Sample	Dilution	Time of exposure		Period of incubation at 37° C.
		½ hr.	1 hr.	
Formalin	1 : 15	Growth	--	4 days
	1 : 10	--	--	"
Cyllin	1 : 100	Growth	--	"
	1 : 50	--	--	"

Eurich found that cyllin 1% will destroy anthrax spores on wool after steeping one hour.

Boiling anthrax spores in water is effective, if sufficiently prolonged.

In the Imperial Health Office at Berlin very resistant anthrax spores on silk were invariably destroyed by three hours boiling; less than three hours gave uncertain results. Further, it was found that the germs which survived boiling were attenuated, as proved by the inoculation of mice; death being delayed to the fourth or fifth day. As men are much less susceptible to anthrax than mice, the danger to the human subject from spores which have been boiled for some time is therefore small. In favour of boiling it can also be said that it is an admirable method of cleansing raw material; and it seems to be largely carried out in Germany both for many varieties of bristles and horsehair. In England, as will be shown, it is impracticable for bristles, and damages horsehair.

Duncan has also shown that though freshly developed anthrax spores can be destroyed by ten minutes in boiling water, yet when contaminated with grease, dirt, and dried animal discharges as in hair, they survive thirty minutes boiling.

With regard to steam, Legge (1906) states that to be effective it must be in contact with the material (in a loosened condition) for a sufficiently long time, and at a sufficiently high temperature; but, in order that the material may not be injured, this temperature must not exceed certain limits. Further, the conditions under which the steam is used, whether saturated or superheated, whether as current or as confined steam, the degree of its pressure and the consequent temperature within the apparatus to which the material becomes exposed, the presence or absence of air, are all of moment in determining efficiency. Disinfection is brought about by the steam coming into contact with a colder surface, i.e. the raw material, on which it condenses, and in so doing gives up its latent heat (sufficient to raise from 15 to 16 times its own weight of wool from 0° F. to 212° F.). Experiments carried

out at Berlin by the Imperial Health Office led to the following conclusions :—

(1) That spores of anthrax on horsehair were destroyed by exposure to current steam for half an hour at 15 atmospheres ( $2\frac{1}{4}$  lbs. =  $218^{\circ}$  F.).

(2) That this method of disinfection if carried out accurately and carefully is practicable for all except white horsehair, and this may be steamed if subsequently it is immediately well washed and bleached ; it is also practicable for some kinds of bristles, i.e. these raw materials are not damaged for the purposes of manufacture ; loosening the bundles was found to make but little difference, but in order that disinfection may be successful it is necessary that :—

(a) The disinfecting machine be of such construction as to secure an even temperature and pressure throughout. The steam should enter from above, then the cold air, being heavier than steam, is pressed out more evenly and the condensation is less. It is inadvisable for the apparatus to stand in the open, as this leads to greater condensation ; it should be warmed before use, and the steam should enter slowly.

(b) In order to prevent damage to the raw materials, the means of controlling temperature and pressure must be accurate; therefore skilled and constant attention is necessary.

(c) Small quantities only (as for example a Russian bale) should be disinfected at a time, in order that the whole of the hair may be exposed to the same temperature.

Many experiments have been carried out in steam disinfection of hair by Webb and Duncan of Worcester (1904). As a result of the first series of experiments, it was found that dust shaken from hair that had been subjected to a temperature of  $245^{\circ}$  F., in a steam disinfecter while bundled, was sterilized. A later series did not confirm this, as dust from carding hair that had been thus steamed still contained spores of anthrax. Hence it was concluded that the hair must be in a loosened condition, or else the steam will not penetrate the bundles. The drier the steam the less damage to the raw material but the less is it likely to destroy the spores. Experience shows that a temperature of  $226-230^{\circ}$  F. is as effective as higher temperatures.

Great care and constant supervision are necessary to secure satisfactory results, and steam cannot be regarded as absolutely certain in effect, though the great bulk of the spores are destroyed, and the vitality of the remainder diminished; after steaming inoculations sometimes took seven or eight days to kill guinea-pigs.

In answer to some questions as to the effect on horsehair of

different methods of disinfection, Mr Webb gave me the following information. It is not strictly true that steaming does not damage horsehair; even in half an hour the elasticity is reduced, and the material becomes more brittle, but not enough to be serious if the quality of the hair is good. Damp heat ruins hair. The dampness of normal pressure would be almost worse than a somewhat higher and drier one.

Steaming white hair turns it slightly yellow, and as it is naturally inclined to this colour, it is depreciated in value, because the yellow colour cannot be removed by subsequent bleaching. Mr Webb's firm disinfects many tons of hair per annum, and his experiments as to steam disinfection, the results of which are briefly given above, exactly corresponded with those of Signor Carlo Pacchetti of Milan; although each worked in ignorance of the other's methods.

Mr Webb says boiling damages the fine ends of hair, affecting the weaving of certain soft classes of hair, causing the ends to break off, and must be excluded as a means of disinfection. The frizzling up of the fine ends of long tail hair in boiling may be due to prior treatment with too strong alkali; this must be reckoned with in any process of disinfection.

Mr Webb states that he has found cyllin in the proportion of 1 in 250 ineffective. In his experiments horsehair was immersed in water in a tub, and the tub was placed in a water bath, which was heated by blowing steam through the water until the temperature in the tub reached 80° F., which favours the germination of the anthrax spores. After eight hours the water in the tub was found swarming with anthrax bacilli. In the place of the water in the tub, horsehair was immersed for twelve hours at the same temperature in a solution of cyllin of varying strength, from 1 in 1000 to 1 in 250; in each case the anthrax bacilli were found diminished in number, but not destroyed. Long immersion of horsehair in water up to 160° F. certainly does not hurt the hair.

Mr Webb's experience of formalin is small; he has found its powers of penetration slight.

Cyllin is now used in various ways by several manufacturers as a disinfectant of horsehair. One London firm immerses horsehair in 1 in 100 cyllin, and water at 66° F., for one hour, the larger bundles being opened out; they state that it does not in any way damage the hair. Another large firm in the Eastern Counties immerses horsehair in 1 in 500 cyllin, heated before immersion to 160°—170° F. The time of

immersion is twelve hours at that temperature, and the weight of hair disinfected each time is 9—10 cwt; they state that stronger solutions gum the hair together; this however, it is said, may be obviated by the addition of a little alkali.

Through facilities kindly granted by a firm of brushmakers, who mainly prepare their own horsehair, it was found possible to carry out some experiments with regard to disinfection. This firm disinfects all horsehair, whatever its origin, by steam, with the following apparatus. A wooden tank is used, holding about 200 lbs. of horsehair, divided by a horizontal grating about two inches from the bottom; steam leaves the boiler at a pressure of 35—40 lbs. to the square inch, and enters the tank below the grating through a tube which has numbers of very fine holes in it. When full of horsehair, and ready for steaming, the tank is covered over with several layers of sacking. Each steaming lasts 20 to 30 minutes.

It was found when the bulb of a maximum thermometer was placed in the centre of the tank, loosely packed in among the bundles, that the highest temperature reached was 220° F.; when tightly tied into a small bundle and again inserted, 218—220° F. The temperature in different parts of the tank varies from 218 to 222° F. These experiments were repeated several times to ensure accuracy. Before the hair is removed the temperature is allowed to fall to 70° or 80° F. When removed the hair is found to be very fairly dry. This process was found effective in destroying anthrax spores artificially placed on bristles, as will be described later. The effect of steaming hair on three consecutive days was tried with the idea that anthrax spores not killed by the first or second steaming would perhaps develop into bacilli, which are killed so much more easily, and would succumb in the third steaming. It was found, however, to damage the hair. Steaming for only thirty minutes was found to make the hair curly and crimped, and increased the difficulty of working it; this would be more disadvantageous in weaving than in brushmaking. Steaming longer than thirty minutes ruined it for all purposes. Further, it is doubtful if in the absence of a suitable medium spores would develop into bacilli on horsehair.

Boiling both white and dark hair in two per cent. potassium perman-ganate for 15 mimutes, followed by subsequent bleaching in sulphurous acid, was tried. It was found that the boiling makes the hair curly and difficult to work; examined while wet nothing wrong was noticed, but when carefully dried the hair was found damaged, being brittle.

Before steaming the hair, sprinkling it with 1 in 100 and 1 in 50 cyllin was tried, using two gallons of the 1 in 100 and one gallon of the 1 in 50 to each tank full of horsehair, i.e. about 200 lbs. The hair was not found in any way damaged commercially, but any larger quantity damaged the hair. Hair immersed in cyllin of a strength 1 in 100 at 176° F. for half an hour was not found to be damaged, though sticky for subsequent working.

Steaming bundles of bristles was found to damage the bristles for some purposes, and also in nine cases out of ten the bundles burst; for this reason alone steaming is impracticable, as the cost of rebundling would be prohibitive even supposing it could be done in England as well, for example, as a Chinaman can do it. Boiling bristle bundles had a similar effect. In order to meet the complaints of the dust from workers on Chinese riflings, dipping both ends of the bundles in paraffin floating on the surface of water has been tried by one firm for some time with advantage. Numerous experiments were made with several liquids of low surface tension, similar to paraffin, in order to find one that would dissolve a disinfectant, carry it up into the bristles, and yet not damage the hair for working; but with slight success. Cyllin as a disinfectant proved useless, preventing the bristles working on the machines; the best results were obtained by making a concentrated solution of carbolic acid in commercial oleic acid, and mixing with paraffin in a strength of 1 in 10 or 1 in 20 of carbolic, but even this was found to make the bristles too greasy to get good results.

Table II contains a description of experiments carried out with reference to the disinfection of anthrax spores. It was found that (1) anthrax spores from an old agar culture were destroyed by steam at 100° C. in half an hour (Experiment 1); (2) anthrax spores artificially placed on bristles were destroyed by exposure to steam for half an hour on three consecutive days; after two days exposure they were much diminished, if not entirely destroyed. None were isolated after the first day's steaming, though anthrax-like bacilli of the A<sup>2</sup> type, being present after the first and second day's steaming, prevented the experiment being absolutely conclusive as to the complete destruction of anthrax spores. These experiments prove, at any rate, that intermittent sterilization is not sufficient to destroy all spores present in bristles; though apparently all anthrax-like spores were eliminated by the third day (Experiments 2, 3, 4). In Experiment 5, anthrax spores artificially placed on bristles were found to be efficiently disinfected when steamed

in the tank described on page 367. The infected bristles were placed in the centre of the tank enveloped in a small bundle of hair, and afterwards were found to be sterile. The temperature was 220° F.

The effect of 1 in 100 cyllin at a temperature of 60° C. with a contact of one hour was tried on old cultures of anthrax containing spores, and old cultures of the three A types. In the first (Experiment 6) the anthrax spores were apparently all destroyed; in the second (Experiment 7) anthrax developed after treatment with cyllin, but growth was much delayed. Of the A types, A<sup>1</sup> was destroyed by the cyllin, but A and A<sup>2</sup> types, though growth was generally somewhat delayed, were certainly not destroyed (Experiment 8). Anthrax-infected bristles treated in a similar manner with cyllin and also with a longer contact, i.e. 1½ hours, appeared to be sterilized, as far as anthrax was concerned; but other bacilli, among them the A<sup>2</sup> type, easily survived the cyllin.

The use of artificial spores (laboratory specimens), in place of natural ones surrounded by grease and dirt making them exceedingly resistant, probably accounts for the different results obtained by Mr Webb in England, and the German Imperial Health Office; for the former used natural spores, and the latter artificial ones on silk, in a similar way that I used them on bristles; this probably accounts for the fact that in my own experiments steam was found effective in getting rid of the anthrax spores, though at the same time in Experiment 5 the steaming in the horsehair tank apparently destroyed spores of the A and A<sup>2</sup> types, certainly as resistant, if not more so, than anthrax spores.

Thus we may conclude that disinfection of horsehair by steam cannot absolutely be relied upon; but that with due care the number of anthrax spores may be diminished, and the vitality of the remainder lowered without appreciable damage to the hair.

That steam is ever likely to be certainly effective in disinfecting horsehair is improbable, since the damper the steam the better chance of destroying the spores, but the greater the damage to the hair; and the drier the steam the less chance of destroying the spores and the less damage to the hair. These antagonistic results produce a deadlock.

For bristles steam is useless as it bursts or loosens the bundles.

Boiling as a method of disinfection is useless, because in the time taken to destroy the spores, 2—3 hours, the material would be considerably damaged.

TABLE II. Disinfection.

No. of Exp.	Material	Apparatus	Disinfectant	Time exposed	Temperature	Method employed	Result	Control Expt.	Remarks and Conclusions
1.	Old agar culture, containing spores of Anthrax.	Anthrax spores in salt solution in test tube heated in autoclave.	Steam in the autoclave at 100° C.	(a) $\frac{1}{2}$ hr. (b) $\frac{1}{2}$ hr. 1st day (c) $\frac{1}{2}$ hr. 1st hr. 2nd hr. 3rd	100° C.	A platinum loop was rubbed over the surface of the old agar culture. An emulsion was made in sterile salt solution after the disinfection, agar plates sown directly (a), (b), (c).	No growth obtained from (a), (b), or (c).	Agar slope inoculated from unheated salt solution showed much typical growth of Anthrax (proved by microscope and cultivation).	The spores were evidently not very resistant.
2.	Bristles artificially infected with Anthrax through tubes.	Bristles placed in dry sterile test tubes, plugged with cotton wool and covered over loosely to prevent any water of condensation entering the tubes.	Steam in the autoclave at 100° C.	(a) $\frac{1}{2}$ hr. (b) $\frac{1}{2}$ hr. 1st day (c) $\frac{1}{2}$ hr. 1st hr. 2nd hr. 3rd	100° C.	After heating in autoclave broth was poured over the bristles. As after incubation Bac. (A.15) of the A <sup>2</sup> type obtained from (a), (b), and (c) became cloudy, agar plates were sown in each case.	No Anthrax was isolated. The colony most like Anthrax was a Bac. (A.15) of the A <sup>2</sup> type obtained from (a). Anthrax was destroyed by 13% steaming. Anthrax-like bac. present from both (a) and (b) but none evident from (c).	Some of the bristles before heating were soaked in warm salt solution and from this agar plate was sown from which Anthrax was isolated, proved by cultivation and microscope.	While not absolutely conclusive without inoculating animals it is probable that most if not all the Anthrax was destroyed by 13% steaming. Anthrax-like bac. both present from both (a) and (b) but none evident from (c).
3.	Experiment 2 repeated again but bristles after heating were spread out on agar plates. No Anthrax was obtained.	The same.	The same.	The same.	The same.	Bristles after steaming, soaked in warm salt solution and agar plates sown.	Salt solution in each case was sterile.	The same.	
4.	Bristles artificially infected with Anthrax through horse's serum as described on page 358.	The same, but bristles were moistened with sterile salt solution before heating.	The same.	The same.	The same.				If it is possible that had the bristles been placed directly upon agar some growth might have been obtained though certainly the number of spores if any that survived must have been much diminished by the steaming.

5.	The same.	Bristles wrapped in paper embedded in bundles of hair of about 2 lbs. wt. which were then tied tightly. Bundles then placed in centre of tank full of hair as described on page 367.	Steam at 220° F. (a) $\frac{1}{2}$ hr. = 104.4° C. Steam leaves the boiler for the tank at a pressure of about 35 lbs. to the sq. inch.	(b) $\frac{1}{2}$ hr. 1st day (18 hrs. interval) $\frac{1}{2}$ hr. 2nd day.	220° F. = 104.4° C. as tested by maximum thermometer. Bulb was placed in centre of a small bundle of hair of about 2 lbs. wt. Bundle then tightly tied and inserted in centre of tank full of hair.	Bristles (a) and (b) found sterile.	The same.	Papers containing bristles after removal from centre of bales found to be damp.
6.	Old agar culture of Anthrax containing many spores.	Anthrax spores in 1 in 100 cyllin in test tube in water bath.	Commercial cyllin 1 in 100.	1 hour.	60° C. temp. of water in water bath.	A platinum loop was rubbed over the surface of the old agar cultures, an emulsion was made in 1 in 100 cyllin in a test tube. After disinfection agar plates sown directly.	No growth could be obtained up to 5 days incubation.	As Experiment 1.
7.	Experiment 6 was repeated in exactly the same manner, after 48 hours incubation growth was obtained on agar which was proved Anthrax by microscope and cultivation, e.g. clear growth in Broth, non-nutritive Bacillus, slow liq. of Gelatine, growth in Gelatine Slab culture, etc.							
8.	Similar experiments to 6 were made using old cultures of Bocilli A, A <sup>1</sup> , A <sup>2</sup> , as examples of the types of Bacilli isolated from bristles; when growth took place it was with one exception found delayed. A and A <sup>1</sup> grew readily, A <sup>2</sup> not at all.							
9.	Bristles artificially infected with Anthrax through salt solution as described on page 359.	Bristles in 1 in 100 cyllin in test tube in water bath.	Commercial cyllin 1 in 100.	(a) 1 hr. (b) 1 $\frac{1}{2}$ hrs.	Temp. varied from 50° C. to 70° C. and one was tried at temp. of the room about 12° C. or 55.5° F.	Agar tubes were stroked directly in each case and also cyllin after disinfection was poured off from bristles, which were then placed in warm sterile Cols. most like Anthrax isolated agar plates sown	In each case and at both temperatures much growth was obtained though unable to isolate any Anthrax.	As Experiment 2.

There remains then immersion of the material in some chemical disinfectant, but up to the present no compound has been produced that can be said to be absolutely effective in destroying the spores without damaging the raw materials. Cyllin at present seems to be the best; the great objection to it is its stickiness, but it is now being used in one or two factories for the disinfection of horsehair. The strength must be not less than 1 in 100 for any chance of success in destroying the spores, and it is doubtful if this is sufficiently strong; but more concentrated solutions render the hair too sticky though it is said the addition of some alkali prevents this. The second point is that the temperature of the disinfecting solution should not exceed 120° F. (or 50° C.) for that strength. The time of immersion must not be less than 1 hour with bales loosened and spread out, but a longer contact is desirable.

Eurich reports favourably on Leach's fluid for disinfecting bales of wool, the strength should be 2 % and the steeping last for an hour.

Further experiments as to the use of formalin under slight pressure and in solution may lead to better results.

Bristles are partly prepared and bundled and it is not practicable in most cases to untie the bundles before use, so that effective disinfection is very difficult; however, from what has been already written, it is evident the danger of infection from bristles is very small, and the method described above of using paraffin, by fixing the dust, should materially diminish even that small risk.

In addition to disinfection of raw materials much may be done to diminish risk by suitable regulations for workshops and factories in which dangerous materials are used.

Table III compares the English and German regulations for the manipulation of horsehair and bristles. There is reason to doubt if the German regulations are properly carried out.

Since the nails frequently act as spore carriers, it is advisable that gloves should be worn in processes preliminary to disinfection, and that the nails should be cut short, and that in washing a disinfectant, as cyllin, should be used with the soap. The use of the nail brush should be insisted on, also a plentiful supply of dry towels to prevent risk of chapped hands, thus leading to further risk. Each factory or workshop should be compelled to provide means for dressing any wounds or abrasions on exposed parts and keeping them covered until healed. The services of a medical man should be retained, in order that any suspicious case may be seen early by him, and, knowing the patient's employment, he may be able to make an early diagnosis, and to apply

the appropriate remedies in time. Also any employee absent from work should be traced by the employer at once, and if the absence is from illness of any kind, the medical man connected with the factory should visit the patient promptly. Respirators for manipulation of undisinfected horsehair are necessary. Mr Webb has known of two cases of internal anthrax and possibly three, and requires men handling raw materials to wear them. Pacchetti also requires his men to use them. Even in the wool trade anthrax recurs in "patches" after long freedom from it.

It is advisable, in issuing regulations, to name one or more disinfectants for personal use, and for disinfecting floors, walls, etc.

Premises of all kinds, however old, are used for factories and workshops, especially for brushmaking. It should be compulsory for employers to provide suitable accommodation, so that dust and waste can be completely removed by wet methods.

The English regulations have not been in force long enough to determine the effect in reducing the number of cases of anthrax, and it is impossible, owing to the absence of notification in Germany, to ascertain definitely if a reduction has taken place there.

In Nuremberg, one of the chief brushmaking towns in Germany, the regulations are carried out, all raw materials being disinfected by steam; yet cases of anthrax still occur, though, considering the great increase in the number of people employed there, relatively less in number.

England and Germany are the only European countries requiring disinfection of raw materials. However, in the works of Signor Carlo Pacchetti of Milan, very large horsehair manufacturers, employing about 700 work-people, steam disinfection of dark horsehair is carried out, while white hair is washed in soda and boiled in potassium perman-ganate, and subsequently bleached according to the German regulations. Also a physician attends every morning to treat accidents and slight ailments, keeping careful watch for appearances or conditions suggesting anthrax, while a surgeon's room is fitted up for the bacteriological investigation of anthrax, and a supply of Sclavo's serum is kept.

Signor Pacchetti states that he has no fear of the disease among his work-people, as he has seen so many successes in dealing with it by the above methods. Cases of anthrax in the earliest stages are caught, treated, and recover, without cessation of work for more than a day or two.

*Industrial Anthrax*

TABLE III.

*Table showing English and German methods and rules for the disinfection and manipulation of Horsehair and Bristles.*

England. Factory and Workshop Act, 1901. Regulations for the use of Horsehair came into force Jan. 1st, 1908.

Germany. Section 120<sup>e</sup>. Industrial code 1902 came into force Jan. 1st, 1903.

Country	Material	Disinfection			Other	Register must be kept, and for each consignment must be signed by the Director of a Bacteriological Lab.	Register must be kept, and for each consignment must be signed by the Director of a Bacteriological Lab.	Register must be kept, and for each consignment must be signed by the Director of a Bacteriological Lab.	Operations previous to disinfection
		Steam	Boiling for ½ hr. in 20% pot. permanganate.	Boiling for 2 hrs.					
England	Nails and manes of horses as imported from China, Russia, and Siberia, whether raw, partially, or wholly prepared.	Steam at 212° F. for ½ hour. Material must be loosened and spread out and fully exposed.	By other means under certificate.	(1) Cert. must be signed by the Director of a Bacteriological Lab.	(1) Wt. of materials.	(1) Cert. must state the method which must be able to destroy Anthrax spores in all parts of the horsehair.	(1) Wt. of materials.	(1) Cert. must be put in Register.	Operations previous to disinfection
Germany	All foreign horse-hair, cowhair or goat-hair, "pigs' bristles and pigs' wool. Erections see below*.	Current steam at 15 atm. (about 220° F.) for 220° F.) for ½ hr.	Potass. permanganate.	Simple boiling for 2 hrs.	Boiling for 2 hrs.	Other methods may be used but they must be approved by the Imperial Chancery.	Register must contain list of exceptions with grounds therefor. Statement of treatment subjected to abroad, also the source, amount, and country of origin of all materials, duration and mode of disinfection, vouchers to be kept, in case of public disinfection.	Unpacking, outfitting from docks, conveyance to apparatus, tying bristles in bundles, sorting for purposes of disinfection, all to be done in special rooms.	Special storing room as above with separate entrance. Room to be kept locked.

Register to be examined each Feb. 1st.

TABLE III (*continued*).

Country	Other operations	7	8	Treatment of dust from operations in col. 7	9	10	11	12	13	14
England	Willowing and dust extracting machines must be covered and provided with efficient screens.		Dust to be discharged in special manner & burnt.	Suitable overalls and head coverings, respirators fresh or cleaned every week to be used for 6, 7, 8, under opening & sorting screens to be cleaned out once a week.	Use of overalls etc.	Provisions as to food	Either meal rooms to be provided or else works must be shut down;	Means of treating wounds and scratches, with hot and cold water, towels, soap, nail brushes, etc.	Lavatory accommodation by employer of Lavatory notice to be placed in the works.	Cautioning notices Age limit No person under 18 allowed to be employed on non-disinfected material.
Germany	Mixing, willowing, and hacking the same as above, sorting and hacking in a special room.		Much the same.	Practically the same except respirators are not mentioned.	The same.	The same.	The same.	The same.	No young person as above.	

- \* (1) If bought already disinfected in the prescribed manner at home or effectually from abroad and if kept separate from non-disinfected material.
- (2) White bristles to be further bleached, or previously bleached as French, if kept separate as under (1).
- (3) Any material which cannot according to present experience be disinfected without serious damage. This being a disputed point forms a considerable loophole for avoiding disinfection.

#### England. Other Regulations.

Lavatory basin or 2 ft. trough to each 5 persons.

N.B.—Draft Nov. 1907 made an exception in favour of white or light grey hair soaked in a warm alkaline solution and wet hacked. Also mentions daily cleansing of the floors.

#### Germany. Other Regulations.

Solid impervious floor rendering removal of dust by wet method easy. Wooden floors to be planed smooth and protected against wet. Walls and ceilings unless washable to be lime-washed annually. New or extension of premises in dusty occupations 530 cub. ft. air to each person.

Moist washing of floors, etc. daily, ventilation of workshops twice a day, burning of coverings of bales, dung or dirt.

Table IV is copied from one by Prof. Ascoli (1906), Physician to Messrs Pacchetti, showing the method of treating and using hair.

#### TREATMENT.

The treatment of anthrax depends on the nature of the disease and the position of the lesion.

The only treatment holding out any hope of success in internal cases is the early intravenous injection of serum; in cases of malignant pustule we may combine local with general treatment including injection of serum.

Local treatment should consist in cauterization or excision whenever possible, and in most cases the former at any rate is possible. Other measures, such as the injection round the pustule of 2% carbolic acid or of iodine, the taking of iodine internally, the use of ipecacuanha both locally and internally are not likely to be of much advantage. Prof. Ascoli strongly recommends cauterization; with a red-hot iron the pustule should be burnt widely and deeply, including a little of the healthy tissues around and below. Excision, except in the case of a very skilful operator, opens the door to possible generalisation. Cauterization is better, though a bad scar would remain in a serious case. In the early stages at any rate no method can vie with this.

The treatment of anthrax by serum (discovered by Sclavo in 1895) has now been tried sufficiently long to enable an idea of its value to be formed. Sclavo (1903) collected figures for cases treated in Italy by the end of 1903 with serum, i.e. 164 cases, 10 deaths, mortality 6.09% as compared with 24.1% for the whole of Italy. Legge, in March 1905, had collected 69 cases with only two deaths; in one of these the injection of serum was delayed, and the second was the subject of other diseases besides anthrax. All but two of these cases occurred in Italy.

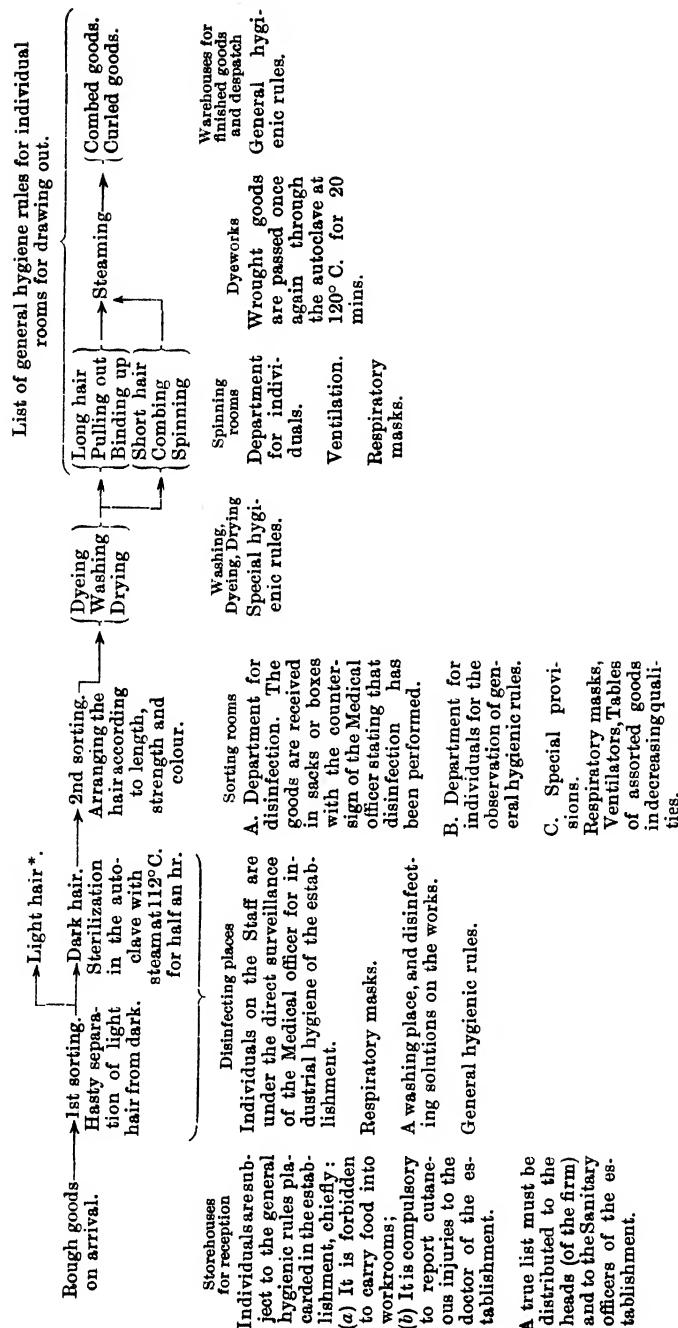
Since 1904 there have been not less than 91 cases in Great Britain treated with serum (Table V), of whom 29 died, per cent. fatal 31.8; 33 of these cases were treated with serum without excision or cauterization, and of these 12 died, per cent. fatal 36.3. The percentage of failures with serum alone decreased in 1906 and especially in 1907, thus:—

Year	Cases	Deaths	
1904	2	1	50 % fatal.
1905	14	7	50 % ..
1906	10	8	80 % ..
1907	7	1	14.3 % ..

kept in  
public disinfection.  
Register to be exam-  
ined each Feb. 1st.

TABLE IV.

*Of the working up of animal hair with relation to the measures of industrial hygiene practised in the establishment of Carlo Pacchetti and Co.*



\* We omit to follow up the manufacture, although intricate, to avoid complicating the diagram. The hygiene measures applied are analogous to those for dark hair.

In 1904 there were 10 cases of anthrax (3 fatal) treated with serum with or without other treatment. Two were treated with serum alone. In one (1) after the injection of an initial dose of 40 c.c., there was rapid improvement but slight scar eventually resulting. In the second (2) the patient was in a hopeless condition at the time of the injection. In a fatal case (4), in which excision and serum formed the treatment, the patient was comatose at the time of the injection and died shortly afterwards. In the third fatal case (5) the patient died 30 hours after excision, having received only 20 c.c.

In 1905 there were 31 cases in which serum with or without other treatment was used. Of these 13 were fatal, 14 were treated by serum alone, and of these 7 died, 4 of the 14 were internal and were all fatal. In two of these internal cases (24 and 40) injections of serum were only made just before death, in the third internal case (27) 40 c.c. were injected, but only 12 hours before death, and in the remaining internal case two injections of 40 c.c. were made, the first 18 hours before death. Of the other three fatal cases, all (external) treated with serum alone, two (15 and 28) were comatose at the time of injection, and died shortly afterwards, and one (25) died from meningeal hemorrhage after 150 c.c. had been injected, during 49 hours of treatment, by subcutaneous and intravenous methods. Of the six fatal cases, in which excision was performed as well as serum injected, one (29) was fatal eight hours after the injection of serum, another (31) 16 hours after injection of serum, and a third (37) five hours after injection of serum; a fourth (20) was injected with serum the day before death, but it was the sixth day of the disease. In the remaining two cases, (13) and (14), 70 c.c. and 80 c.c. respectively failed to save life, though treatment lasted 51 and 36 hours, commencing on the second day of the disease in each case.

Three cases (16, 19, and 36) that recovered developed slight secondary rashes; in two other cases (21 and 38) there was much sloughing of tissue, despite the injection of serum. In a case (33) at the time of the injection, anthrax bacilli were cultivated from the pustule; 19 hours after the injection they had absolutely disappeared. In several cases, notably (17), (19), (22), (33), (39), (41), rapid improvement after injection of serum was very marked.

In 1906 of 27 cases treated with serum 9 were fatal; 10 of these were treated with serum alone with 3 deaths. There were 3 internal cases, and injections of 200 c.c. probably saved the life of one man, as the case (66) was severe; it must be remembered, however, that no bacterio-

logical examination was made. In the second of the internal cases (65) the serum treatment seemed at first to cause much improvement, when a sudden collapse ensued followed by a fatal issue. In the third internal case (67), in spite of injections of 80 c.c. subcutaneously, and 50 c.c. intravenously, there was no reaction; death ensued within 60 hours of the first injection.

In case (58) there was ulceration in the intestines as well as a malignant pustule, probably due to separate infections, as no other parts were affected; and 20 c.c. of serum had no effect. In case (52) there was similar ulceration, and the injection of serum was not made until 14 hours before death. Of four other fatal cases, two (53 and 64) died within 24 hours of treatment commencing. The third (45) was evidently moribund when the injection was made; and the fourth (44) had two pustules, and only received 20 c.c. though 10 of them were injected intravenously. In case (49) there was marked and rapid improvement after injection, though the case was a severe and unfavourable one.

Another patient (62) recovered after 190 c.c. had been injected, and in (68) there was rapid improvement after injection of 80 c.c.

Sufficient details are lacking as to the cases treated with serum in 1907. 23 cases in all were treated with serum, with or without other treatment; four died. Of these cases, seven, of which one died, were treated with serum alone; in this fatal case (80) death was attributed to pneumonia.

In case (77) 100 c.c. failed to save and in case (88) the disease was probably too far advanced owing to faulty diagnosis for the injection to be of much value. Case (79) was a severe one, but improvement followed the injection.

Thus it will be seen that the mortality of cases treated with serum in England is very considerably higher than that of those Italian cases collected by Sclavo (1903) and Legge; and is also higher than the mortality of all cases of English Industrial Anthrax, previous to the introduction of serum, i.e. previous to 1904. Thus:—

Mortality of all cases treated with serum in this country	= 31·8
Mortality of all cases treated with serum only in this country	= 36·3
Mortality of Sclavo's (1903) cases of serum treatment	= 6·09
Mortality of Legge's cases of serum treatment	= 2·9
Mortality of cases previous to the introduction of serum (all cases of Industrial Anthrax, England 1889—1903)	= 26·5

How are these differences to be explained? Possibly as follows:—

Examining in detail the cases treated with serum during the years 1904 and 1905, because of the fuller details of cases in those years, seven treated with serum alone were either comatose, or in a hopeless condition, when the first injection of serum took place, and died within 24 hours of the first injection. In five cases, treated with excision and serum combined, the disease was similarly far advanced; while one case treated by combined excision and serum only received 20 c.c. of the latter, and another died from meningeal hemorrhage, making a total of 14 cases. Now in 1904 and 1905, there were 41 cases in which the serum treatment was used with 16 deaths; and if we deduct, as we fairly may, the above 14 fatal cases, which were either dying at the time of injection, or in one case about to die of a complication, we have 27 cases with two deaths treated by serum alone, or in conjunction with other methods, in 1904 and 1905. This gives a percentage mortality of only 7·4, which closely corresponds with Sclavo's (1903) figures. Among ~~Sclavo's (1903) cases~~ <sup>Sixty</sup> cases were only two or three in which treatment was commenced when in a hopeless condition. His fatal cases being only 10, the mortality of 6·09 would have been considerably lower had these been excluded.

Further, undoubtedly an insufficient amount of serum was injected, especially in the earlier cases; and it will be noticed that an increase in the amount injected corresponds to a fall in the mortality from 50% in 1904 to 14·3 in 1907.

The way in which the serum acts is disputed. Sclavo (1903) regards it as stimulating the defensive activity of the phagocytes, an anti-bacterial rather than an antitoxic action. Andrewes, from observations of case (33), infers that the serum exerted a powerfully bactericidal action; increase of the local oedema, after injection, possibly depended on the liberation of an intra-cellular toxin from the disintegrated bodies of the bacilli, which 19 hours after the injection could not be cultivated from the pustule. Prof. Ivo Bandi (1904), from observation of two successful cases treated with a serum of his own prepared in a very similar manner to Sclavo's (1903), concludes that the action is anti-bacterial, because of the immediate arrest of the progressive invasion of the organism by the bacillus, and antitoxic, because of the sudden improvement in the general condition and the complete and immediate restitution of the renal function.

Sclavo's (1903) claims as to the effects of anti-anthrax serum may be summarised as follows:—

(1) Anti-anthrax serum even in very large doses is innocuous and can be well borne even when introduced into the veins.

(2) No case taken in an early stage or of moderate severity is fatal if treated with serum.

(3) With serum some cases are saved when the condition is most critical, and the prognosis almost hopeless.

(4) When injected into the veins the serum quickly arrests the extension of the oedematous process so as to reduce notably the danger of suffocation, which exists in many cases where the pustule is situated on the face or neck.

(5) The serum, if used soon enough, reduces to a minimum the destruction of the tissues where the pustule is situated, and thus avoids creating deformity.

(6) In some situations of the pustule, as the eye-lid, serum must be used in preference to any other treatment, it being the only one which holds out hope of success without permanent injury, and in cases of internal anthrax the early injection of serum intravenously is the only remedy likely to be successful.

The serum may be obtained from Elia Coli of Siena in Italy in 10 c.c. tubes. 30 or 40 c.c. should be injected in 3 or 4 lots under different parts of the skin of the abdomen, the usual precautions as to sterilizing the skin and syringe being observed. After 24 hours, if there has been no improvement, either in the general or local conditions, further injections of 20, 30, or 40 c.c. should be made.

In severe cases 10 c.c. may be injected into a vein on the back of the hand and repeated in 2 or 3 hours. A rise of temperature following the injection is to be regarded as a favourable indication. Sometimes a rash develops with or without febrile symptoms 3 to 8 days after treatment has commenced. This is not peculiar to the anti-anthrax serum, but may occur after injection of any other kind of serum. In the rare instances where it occurs, it is unimportant. The serum will keep for two years; if sterile and kept in a dark and cool place it will not lose its efficacy, though a slight deposit may occur. Sclavo has since advocated much larger initial doses injected subcutaneously, and it is probable that better results would be obtained if 100 c.c. were first injected, followed, after an interval sufficient for the body to re-adjust itself to the altered conditions, by other injections.

TABLE V.

*Cases of Anthrax treated with Serum*

No. of Case	Year	Locality	Sex	Age	Industry	Occupation	F = Fatal	Rm = Recovery slight	Rs = Recovery severe	Nature of Anthrax and situation of pustule	Verification	
1	1904	London	M	31	Horsehair	—	—	Rm	—	Malar eminence of cheek	Culture, etc.	—
2	"	—	—	—	—	—	F	—	—	Malignant Anthrax oedema	—	—
3	"	Kidderminster	F	35	Woollen	Husband wool sorter	—	—	R	Right angle of mouth	Culture, etc.	—
4	"	N. Stafford	—	—	—	—	F	—	—	Hand	—	—
5	"	—	—	—	—	—	F	—	—	Neck	—	—
6	"	Bradford	—	—	—	—	—	—	R	—	—	—
7	"	"	—	—	—	—	—	—	R	—	—	—
8	"	London	—	—	—	—	—	—	R	—	—	—
9	"	—	—	—	—	—	—	—	R	—	—	—
10	"	—	—	—	—	—	—	—	R	—	—	—
11	1905	Bradford	M	61	Wool combing	Woolrunner	F	—	—	Internal (pulm.)	—	—
12	"	"	M	40	"	Opening bales	—	Rm	—	Neck	Doubtful case	—
13	"	"	M	47	"	"	F	—	—	Neck	—	—
14	"	"	F	36	"	Opening, untying knots	F	—	—	Cheek, rapid generalisation	—	—
15	"	"	F	42	"	Finishing boxwinder	F	—	—	Under chin	—	—
16	"	Bingley	M	35	Mohair spinning	Combing manager	—	—	Rs	No pustule, much oedema, right eyelid and face	No Anthrax Bacilli found on culture	—
17	"	Dewsbury	M	30	Blanket making	Blender, etc.	—	Rm	—	Left cheek	—	—
18	"	Kidderminster	F	18	Spinning & carpet manuf.	Roving frame near carding machine	—	Rm	—	Left forearm	—	—
19	"	London	F	25	Boot manfg.	Lining upper leathers	—	Rm	—	Cheek, typical 2nd pustule	Anthrax Bac. not found on culture	—
20	"	Wellingboro'	M	31	"	Heelbinder	F	—	—	Neck, much oedema of chest	—	—
21	"	Bradford	F	25	Wool combing	Opening fleeces	—	—	Rs	Left upper eyelid, great oedema of face	Anthrax Bac. found	—
22	"	Kidderminster	F	?	Wife of woolcomber	→	—	Rm	—	Chin	—	—
23	"	Bradford	F	42	Wool combing	Washbowl	—	—	Rs	Neck	—	—

TABLE V.

in England to the close of 1907.

Possible source of infecting material	Treatment E.=Excision S.=Serum	Remarks. Particulars as to Serum, etc. (Serum injected subcutaneously unless otherwise stated)	Probable stage in days when case came under treatment	Various
—	S.	40 c.c., glands involved, inj. followed by slight rise of temp. and increase of oedema, rapid improvement. Eschar separates on 17th day, slight scar.	4th	B.M.J. Jan. 7, '05.
—	S.	30 c.c. inj. when patient comatose 4 hours before death.	10th	Lancet, March 25, '05. Legge, Milroy Lect.
Husband working on Persian	S. & E.	30 c.c. inj. in 4 lots on 5th day, discharged on 8th with healthy wound.	5th	Lancet, Feb. 4, '05.
—	S. & E.	Patient comatose when serum injected.	—	Lancet, March 25, '05. Legge, Milroy Lect.
—	S. & E.	2 injections of 10 c.c., death 30 hours after excision.	—	Ditto.
—	S. & E.	Rapid recovery.	—	Ditto.
—	S. & E.	Ditto.	—	Ditto.
—	S. & E.	Ditto.	—	Ditto.
—	S. & E.	Ditto.	—	Ditto.
—	S. & E.	Ditto.	—	Ditto.
Camel and Goat	S.	40 c.c. rept. same day, total 80 c.c., fatal 18 hours after treatment commenced.	3rd	—
Camel, Cow, Goat	E. & S.	40 c.c. on 21st day.	21st	—
Camel hair, E. Indian	E. & S.	40 c.c. on 1st day treatment, 40 c.c. on 2nd day treatment. Fatal within 51 hrs. of commencement of treatment.	2nd	—
Van Mohair, Persian	E. & S.	40 c.c. rept. once on 2nd day of disease, 40 c.c. on 3rd day, 30 c.c. 4th, fatal within 36 hours of treatment.	2nd	—
Camel hair and foreign grey wool	S.	40 c.c. on 2nd day of disease, patient then comatose, fatal shortly afterwards.	2nd	—
Van and Cape Mohair & Alpaca	S. carbolic	2½ % carbolic repeatedly injected, 40 c.c. 2nd or 3rd day of disease, 30 c.c. next day, improvement. Later developed pains in arms and legs and slight rash.	1st & 2nd	4 mos. after still unable to work & a yr. from the disease suffers from much oedema of face & neck.
E. Indian	E. & S.	3rd day excision, 4th 40 c.c. followed by slight rise of temperature, 5th day normal, discharged 17th.	3rd	Lancet, 1905, Vol. II. p. 1329.
Persian	E. & S.	5th day E. and 40 c.c.	5th	—
Tanned leather	S.	10 c.c. 2nd day of disease, 3rd day 10 c.c. every 2 hours up to 40 c.c., 4th day 20 c.c., marked improvement after injections, about 14 days later slight scarlet rash.	2nd	—
,	E. & S.	E. 4th day of disease, serum 70 c.c. in 2 doses 6th day, fatal 7th day.	4th	—
Persian	S.	20 c.c. on 3rd day of disease, 10 c.c. on 4th, 50 c.c. 5th, 20 c.c. 6th, general condition improved after 2nd injection and oedema began to be reduced. Result, recovery with much sloughing and ectropion subsequently improved by a plastic operation.	3rd	B.M.J. 1905, Vol. II. p. 118.
Husband working on Persian	E. & S.	30 c.c. on 3rd day of disease, discharged on the 8th day.	3rd	Lancet, 1905, Vol. I. p. 992.
Probably Persian	E. & S.	10 c.c. on 5th day of disease, 10 c.c. 6th, discharged 18th day.	5th	—

TABLE V (*continued*).

No. of Case	Year	Locality	Sex	Age	Industry	Occupation	F = Fatal	Rm = Recovery		Nature of Anthrax and situation of pustule	Verification
								slight	severe		
24	1905	Bradford	M	22	Wool combing	Card feeding	F	—	—	Anthracoëmia, no ext. lesion	Anthrax Bac. found in blood before death
25	"	"	—	57	"	Top packer and warehouse	F	—	—	Forearm	—
26	"	"	—	37	Worsted spinning	Sorting	—	—	Rs	Upper arm, glands affected	—
27	"	Saltaire	M	45	Wool spinning	Sorter	F	—	—	Internal (pulm.)	—
28	"	E. London	M	42	Hides Docks	Dock labourer	F	—	—	Neck, oedema of chest, delirium	Anth. Bac. not found before or after death
29	"	London	M	47	Fur & skin warehouse	Sorter and packer	F	—	—	Neck, much swelling	—
30	"	Worcester	M	33	Hides	Liming in Tan-yard	—	—	Rs	Neck, oedema of chest, great collapse	—
31	"	Liverpool	M	44	Hides Docks	Dock labourer	F	—	—	Neck, great oedema of chest	—
32	"	London	M	35	Hides Wharfingers	Sorter	—	Rm	—	Neck	—
33	"	"	M	30	Horsehair dressing	Drawer of hair	—	Rm	—	Forehead, following injury, enlarged glands	Cultures and inoculation
34	"	Queensbury	M	42	Mohair spinners	Packer	—	Rm	—	Left cheek	—
35	"	Bradford	M	41	Wool combing	Willowing	—	Rm	—	Left eyebrow	—
36	"	"	F	26	"	Card feeder	—	Rm	—	Neck	—
37	"	"	F	?	"	Comb minder	F	—	—	Neck	—
38	"	Bingley	M	30	"	Washer	—	—	Rs	Hand and arm	—
39	"	Liverpool	F	17	Woolbroker	Sorter	—	Rm	—	Forehead	—
40	"	Bradford	M	41	Wool combing	Card jobber	F	—	—	Internal (pulm.)	—
41	"	Kidderminster	F	16	"	Spinning	—	—	Rs	Cheek	—
42	1906	Bradford	M	37	"	Willeyer	—	Rm	—	Neck	Microscope, Anthrax Bac. found in pustule
43	"	"	M	51	"	Washbowl feeder	—	Rm	—	Elbow	Anthrax Bac. not found in pustule
44	"	London	M	31	Horsehair manuf.	Dresser	F	—	—	Neck & forehead, 2 pustules, Anthrax septicaemia	Culture, etc.

TABLE V (*continued*).

Possible source of infecting material	Treatment E = Excision S = Serum	Remarks. Particulars as to Serum, etc. (Serum injected subcutaneously unless otherwise stated)	Probable stage in days when case came under treatment	Various
Persian only	S.	30 c.c. on 8th day of disease, 4 hours before death, only when comatose.	8th	—
Persian chiefly	S.	40 c.c. on 3rd day of disease, 35 c.c. on 4th, 40 c.c. subcut. and 35 c.c. intravenously on 5th, fatal 49 hrs. after treatment commenced, total S. injected 150 c.c.	3rd	Death due to Meningeal hemorrhage on 8th day of disease.
Angora Mohair	E. & S.	E., & S. 20 c.c. on 2nd day of disease, 20 c.c. on 3rd, 30 c.c. on 4th. Temp. normal after 4 days treatment.	2nd	—
Turkey Mohair, Alpaca ?	S.	40 c.c. on 5th day of disease, fatal 12 hours later.	4th	—
Goods shipped through Oporto	S.	20 c.c. on 7th or 8th day, condition nearly hopeless when treatment began.	7th or 8th	—
American wolf, Australian opossum, China fox	E. & S.	E. 4th day of disease, S. 5th day, tracheotomy, fatal 8 hours after injecting serum.	4th	—
Morocco and Calcutta goat skins	E. & S.		4th	—
West African	E. & S.	30 c.c. 5th day of disease, no reaction, fatal 16 hours later.	4th	—
Elk hides, Straits Settlements	E. & S.	30 c.c.	23rd	—
Hair of all sorts except China manes	S.	40 c.c., no rise of temp. following injection, but for 2 days oedema increased, eschar separates 18th day, leaving hardly any scar, no Anthrax Bacilli could be cultivated from pustule 19 hours after injection.	3rd or 4th	B.M.J. 1905, Vol. 1. p. 296.
Turkey and Cape Mohair	E. & S.	40 c.c. on 6th day, discharged 18th day.	6th	—
Alpaca Mohair and Camel hair	Carbolic S.	40 c.c. on 3rd day of disease, discharged 13th day.	3rd	—
Persian locks	E. & S.	40 c.c. 2nd day of disease—secondary measles-like rash, joint pains, temp. etc. lasting 2 days after 14 days interval, temp. (after injection of serum) 105° F.	2nd	—
Brown Persian and Egyptian	E. & S.	40 c.c. on 5th day of disease, comatose, died 5 hours later.	5th	—
Van, Persian Mohair	S. Carb. Inj. 2½ %	Very severe. Carb. inj. rept. every 4-6 hours for 4 days. 40 c.c. 3rd day, 40 c.c. on 4th day of disease—much sloughing and subsequent deformity improved by later plastic operations.	3rd	—
E. Indian Camel hair	E. & S.	20 c.c., oedema: general condition rapidly improved.	4th	—
E. Indian Alpaca	S.	40 c.c., patient in a state of collapse when serum injected, died 6 hours later.	4th	—
Persian	S.	40 c.c., oedema diminished in 24 hours, discharged 5th day after injection of serum.	3rd or 4th	—
Russian camel, E. Indian goat, Turkey & Cape Mohair	E. & S.	3 injections of 10 c.c.	—	—
Van, Turkey & Cape Mohair, English & Colonial	S.	30 c.c.	—	—
China	E. & S.	10 c.c. subcut. and intravenously, both pustules excised.	—	—

TABLE V (*continued*).

No. of Case	Year	Locality	Sex	Age	Industry	Occupation	F = Fatal	Rm = Recovery slight	Rs = Recovery severe	Nature of Anthrax and situation of pustule	Verification
45	1906	Wellingboro'	M	21	Brush-making	Pan hand	F	—	—	Lower maxilla, much oedema	Microscope
46	"	Long Melford	M	17	Horsehair manuf.	Hackler and opener of bales prior to disinfection	—	Rm	—	Neck (fever)	"
47	"	Liverpool	M	49	Hides Stevedoring	Dock labourer	—	—	Rs	Neck	"
48	"	London	M	27	Hides Wharfinger	Labourer handling hides	—	—	Rs	Forehead, glands affected	Anthrax Bac. not found
49	"	Liskeard	M	21	Tanning	Dressing carcass of animal dead from Anthrax	—	Rm	—	Beneath chin, size of a 5/- piece	Microscope
50	"	London	M	31	Hides Docks	Loading hides	—	—	Rs	Nape of neck	Culture, etc.
51	"	"	M	—	Hides	Furrier (occupier)	—	R?	—	Outer Canthus	"
52	"	"	M	41	Wharfinger	Stevedore	F	—	—	Side of neck, secondary intestinal	"
53	"	Liverpool	M	29	Hides Stevedoring	Dock labourer	F	—	—	Shoulder	Microscope
54	"	Penrhyn	M	38	Bone manure	—	—	Rm	—	Back of neck	—
55	"	Gomersal	M	17	Blanket & Carpet Yarn	Waste shaker	F	—	—	Neck	Culture
56	"	Liverpool	M	35	Woolstores (warehouse)	Labourer loading cart	—	Rm	—	Right cheek	Microscope
57	"	Bingley	M	17	Mohair spinning	Noil picker	—	Rm	—	"	"
58	"	"	M	58	Wool combing	Wool washer	F	—	—	Left wrist, secondary intest.	Culture, etc.
59	"	Kidderminster	M	16	Carpets, etc.	Spinner in carpet factory	—	Rm	—	Arm and foot, 2 pustules	Culture
60	"	Bradford	M	25	Wool combing	Willeying & card jobbing	—	Rm	—	Right cheek	Microscope
61	"	Dewsbury	F	32	Blankets and rugs	Weaver	—	Rm	—	Left eyebrow, oedema of face	"
62	"	Saville Town	M	23	Yarn and spinners	Willeyer	—	—	Rs	Upper eyelid, much oedema, severe case	Culture, etc.
63	"	Bradford	F	26	Wool combing	Finisher	—	—	Rs	Left side of neck	Microscope, Anthrax Bac. found in pustule but not in blood
64	"	Queensbury	F	17	Spinners of Alpaca & Mohair	Spinner	F	—	—	Chin, much oedema of neck	Microscope
65	"	Bradford	M	42	Wool combing	Wool puller and runner	F	—	—	Internal pulm., pleural & pericardial effusions	Culture, etc.
66	"	"	M	53	"	Finishing Box minder	—	—	Rs	Internal (double pleural eff.)	Not verified bacteriologically

TABLE V (*continued*).

Possible source of infecting material	Treatment E = Excision S = Serum	Remarks. Particulars as to Serum, etc. (Serum injected subcutaneously unless otherwise stated)	Probable time in days from first case under treatment	Various
China & Siberian bristles	E. & S.	10 c.c., temp. was 103·4° F., pulse 120. General appearance bad, face dusky when first seen.	—	—
China & Siberian	S.	—	—	—
Dry Arabian hides	E. & S.	60 c.c.	—	—
China & Thibet	S.	—	—	—
Carcase of horse dead from Anthrax	E. & S. carbolic	General condition bad when first seen, 8 hours interval after excision and injection of carbolic before serum injected, 12 hours after injection of serum great improvement.	—	—
Buffalo hides from Penang	E. & S.	—	—	—
Russian wolf	E. & S.	60 c.c.	—	—
?	E. & S.	Death within 24 hours of admission. Stomach and small intestines ulcerated. Serum was only injected when in a state of collapse 14 hours before death.	—	—
Dry Rangoon hides	E. & S.	Fatal within 24 hours of admission.	—	—
Local bones	E. & S.	30 c.c., 24 hrs. after excision and carbolic 5 %, 24 hrs. after injection of serum wound looked healthy.	—	—
E. Indian, Chinese	E. & S.	—	—	—
Goathair, E. Indian & Persian wools	E. & S.	—	—	—
Mohair	S. inj. of 2½% carb.	40 c.c.	—	—
All classes of Cape & Turkey	S.	20 c.c. Internal changes practically confined to bowels. Probably therefore swallowed some Anthrax spores.	—	—
Persian	E. & S.	—	—	—
Persian camel	E. & S.	40 c.c.	—	—
E. Indian, Bradford waste	S.	40 c.c.	—	—
E. Indian, Scotch & native	S. inj. of carbolic	190 c.c. at intervals.	—	—
Van Mohair	E. & S.	40 c.c.	—	—
Turkey & Cape Mohair	E. & S.	2 injections of 40 and 80 c.c., fatal within 24 hours of admission.	—	—
Persian & Turkey Mohair	S.	40 c.c. repeated 3 times, improvement after the first 3 doses, then a sudden collapse.	—	—
Persian	S.	200 c.c.	—	—

TABLE V (*continued*).

No. of Case	Year	Locality	Sex	Age	Industry	Occupation	F = Fatal	Rm = Recovery slight	Rs = Recovery severe	Nature of Anthrax and situation of pustule	Verification Culture
67	1906	Bradford	M	49	Wool combing	Card minder	F	—	—	Internal (pulm.)	
68	"	Kidderminster	F	26	Worsted spinning	Comb minder	—	—	Rs	Left side of face	Culture, etc.
69	1907	Liverpool	M	54	Hides and skins	Dock labourer	F	—	—	Neck	Microscope
70	"	Frome	M	35	"	"	—	R	—	Right eye	Culture, etc.
71	"	Frome	M	48	"	Lime pit foreman	—	R	—	—	—
72	"	Liverpool	M	32	"	Dock labourer	—	R	—	Neck	—
73	"	S. London	M	27	"	"	—	R	—	Left side of neck	—
74	"	E. London	M	40	Wool	Wheeling bales of wool in trucks	—	—	R	Cheek	Culture, etc.
75	"	Earlsheaton	F	23	Blanket factory	Scribbler feeder	—	—	R	Forehead	"
76	"	Ravensthorpe	F	14	Wool	Yarn hank winder	—	—	R	Forearm	"
77	"	Bradford	M	48	"	Wool washer	F	—	—	Left wrist	—
78	"	Kidderminster	M	31	"	Combwinder	—	—	R	Right arm	Microscope
79	"	Kidderminster	F	17	"	Finisher in spinning department	—	—	Rs	Right cheek bone	"
80	"	Bradford	M	56	"	Warehouseman	F	—	—	Left eye	Culture, etc.
81	"	"	M	23	"	Card grinding	—	—	R	Ear	"
82	"	Liverpool	M	41	"	Tearing canvas covers of bales of wool	—	—	R	Cheek	"
83	"	Kidderminster	F	26	"	Wool spinner	—	—	R	Eyelid	Microscope
84	"	Liverpool	M	39	Wool warehouse	Dock labourer	—	—	R	Left cheek	—
85	"	Kidderminster	F	22	Wool	Card feeder	—	—	R	Hand	Culture, etc.
86	"	Liverpool	F	19	Horsehair	Carrier	—	—	R	Left side of face below ear	—
87	"	London	M	44	"	Drawer	—	—	R	Neck	Culture, etc.
88	"	Liverpool	F	53	"	Forewoman hair sorting	F	—	—	Upper lip	Microscope
89	"	"	M	46	"	Drawer & wet hackler	—	—	R	Left cheek	"
90	"	Folkestone	M	53	"	Plasterer	—	—	R	Right eyebrow	"
91	"	N. London	F	?	"	Niece of worker in horsehair	—	—	R	Neck	Culture, etc.

TABLE V (*continued*).

Possible source of infecting material	Treatment E.=Serum	Remarks. Particulars as to Serum, etc. (Serum injected subcutaneously unless otherwise stated)	Probable stage in days when case came under treatment	Various
Persian sample proved to contain Anthrax spores	S.	Injections of 40 c.c. subcut. and 50 c.c. intraven. and 40 c.c. subcut. Influenza bronchitic type, no reaction to serum.	—	Death after 60 hours of treatment.
Persian	E. & S.	2 of 40 c.c. Rapid improvements.	—	—
Chinese hides	E. & S.	—	—	—
"	E. & S.	—	—	—
Indian, Morocco, Algiers	Cautery, carb. ac., serum	—	—	—
Wet Italian hides	S.	—	—	—
Elk hides from Bangkok	E. & S.	—	—	—
Colonial	E. & S.	—	—	—
Various foreign wools from Europe & Asia	E. & S.	40 c.c.	—	—
Indian goat, British cow	E. & S.	—	—	—
Russian & E. In- dian hair, camel, goat & lamb	E. & S.	100 c.c.	—	—
Australasian	E. & S.	—	—	—
Persian	S.	Great oedema. Constitutional symptoms subsiding after injection of serum.	—	—
Cape & Turkey Mohair	S.	Death attributed to pneumonia accompanied by local Anthrax.	—	—
Persian	E. & S.	—	—	—
E. Indian wool and goat hair	E. & S.	2 injections of 20 c.c.	—	—
E. Indian and English	S.	—	—	—
E. Indian wool and goat hair	E. & S.	—	—	—
E. Indian, Colonial	E. & S.	40 c.c.	—	—
Probably Russian hair	E. & S.	—	—	—
Siberian hair	S.	40 c.c.	—	—
Russian hair	E. & S.	Case not diagnosed at first, as symptoms not typical.	—	—
"	E. & S.	—	—	—
? origin of hair	S.&carb. ac. 10%	—	—	—
—	E. & S.	Patient's neck accidentally scratched by her uncle's finger.	—	—

**SUMMARY.**

The result of bacteriological research indicates that the material infected is probably not large compared to the total amount used, and that in suspected samples of both hair and bristles anthrax bacilli can be isolated either by inoculation of animals or by plate cultivation.

Difficulties are met with in the inoculation of animals owing to the presence of the bacillus of malignant oedema, which, unless special methods are used as shown by Duncan, will often mask the presence of the anthrax bacillus altogether.

In separation by means of agar plates the presence of bacilli very closely resembling anthrax again leads to error. Three types of these bacilli have been met with in hair and bristles; viz.: *Bac. A<sup>2</sup>* or *Bac. anthracoides* of Bainbridge, possibly the same as *Bac. subtilis*, the *Bac. A<sup>1</sup>*, and *Bac. A*. The last two do not exactly correspond with any known bacilli, and so far as we know at present are of little importance, but in view of the experiments of Gilruth showing that guinea-pigs, rabbits, and sheep can resist the inoculation of large doses of virulent anthrax bacilli completely, provided these organisms are mixed with a larger quantity of some other organisms which are non-pathogenic to these animals, and that a small amount of immunity to pure anthrax is conferred, it is certain that the presence of these anthrax-like bacilli, evidently closely related to the anthrax bacillus, cannot but be of benefit; and it is possible that further experiments may show that injections of them mixed with cultures of anthrax bacilli of varied virulence may confer a higher degree of immunity than do mixtures of anthrax with other non-pathogenic bacilli, as shown by Gilruth (1904).

Webb has observed during experiments in disinfection with cyllin that there was an apparent decrease of anthrax bacilli while the bacillus of malignant oedema increased; which suggested to him that the two are antagonistic. If this is so, is it possible to eliminate the anthrax bacillus from horsehair by increasing the quantity of an antagonistic bacillus, whether that of malignant oedema or other?

The fact that practically all bristles and horsehair on arrival in this country are centred for a time in two or three London warehouses raises the question whether it would not be possible to disinfect the material before distribution. Were disinfection thus centralized it would be a comparatively simple matter to protect the limited number of people exposed to risk in cutting the knots of the bundles and

spreading the horsehair out for disinfection; then the necessity for formal regulations in horsehair and brushmaking factories and workshops in a great measure would be obviated. The manufacturers would gain in being freed from risk of anthrax among their employees and, further, would be able to use hair that many of them have preferred to discard on account of its dangerous properties.

The risk of infection from bristles is so small and the difficulty of disinfection so great on account of its bundled condition that with our present knowledge central disinfection for this class of material cannot be recommended, especially as by such simple devices as the use of small quantities of paraffin this small risk may be still further diminished.

In the case of horsehair it would be necessary, if steam were the disinfectant, to separate the white from the dark hair prior to disinfection. There would be very considerable objection on the part of the trade to central disinfection of horsehair because of the increased cost; many of the manufacturers have already gone to the expense of erecting their own disinfecting plant; moreover, by most disinfecting processes hair is so easily damaged.

From what has been written about disinfection it is evident that at present there is no method of destroying with absolute certainty spores of anthrax in horsehair and bristles, but by the use of steam under slight pressure the risk to the human subject arising during the various manipulations may be diminished very considerably. It has been suggested by Duncan that exposure of infected material to a temperature of about 98° F. for a short time would favour the development of spores of anthrax into bacilli, which then might be easily destroyed by a further short exposure to steam under pressure. This practically amounts to intermittent sterilization. Experiments already described indicate that most if not all the anthrax bacilli were destroyed by this method, but that the bristles were not rendered absolutely sterile; the spores of anthrax, being laboratory specimens artificially placed on bristles, were more easily destroyed than those under natural conditions would have been. It is therefore probable that, as the medium is unsuitable, development of anthrax spores would not take place, and that intermittent sterilization would not be absolutely effective; moreover the exposure of the material to damp steam at normal pressure or steam of any sort for more than half an hour on one occasion damages the hair considerably. That steam is ever likely to be made more effective than at present is improbable, since the dampness of steam at

low pressure damages the hair, but is more likely to effectually destroy the spores, while the drier the steam, the more damage to the hair, but the more likely are the anthrax spores to be destroyed. More hope in the future lies in the use of liquid disinfectants; while none of these up to the present has been found certainly efficacious, cyllin in a strength not exceeding 1 part in 100 of water and at a temperature below 50° C. (122° F.) does not damage hair. Eurich speaks favourably of formalin solution and Leach's fluid in the disinfection of woollen bales.

The possibility of placing a check on the importation of infected raw materials must be considered. In Italy certificates of origin have been found of very little service. The Foreign Office might possibly issue a list of regulations and instructions which, if they could be efficiently enforced, would prevent the goods from entering this country. The objection of increased cost at once arises if, for example, a further dressing, sorting and bundling of the dangerous mane hair from China were required so that it should enter this country half prepared, as do bristles; on the whole it is improbable that measures on these lines would be very effective.

With regard to the measures introduced in Germany a few years ago, and quite recently in England, more stress might be laid on the necessity of washing, use of nail brush, keeping the nails short; in washing the use of an efficient disinfectant is advisable; for this purpose cyllin does admirably, being compatible with soap. Experience shows that soap and water are the true safeguards after handling infected material, and those who use the same stuff after disinfection should wash hands, face, and neck before going home to a meal. By these means, too, the likelihood of workers carrying infection outside would be diminished. The ignorance and carelessness of the workers are undoubtedly factors in the spread of anthrax. The use of overalls and gloves, though unpleasant and disliked by the work-people, yet is very necessary as cases quoted show.

The regulation by the Home Office requiring the employer to exclude persons suffering from cuts and scratches is, to all intents and purposes, a dead letter. It is impossible to carry out. Anthrax seldom has begun from a recognised scratch or cut; almost every case has undoubtedly arisen from scratching with the nails, where there has been no previous cut but some irritation either by dust or by some insect.

Recent decisions show that anthrax is classed as an accident coming within the scope of the Workmen's Compensation Act.

Facilities for bacteriological examination given by the Home Office since 1899 for verification of doubtful cases might with advantage be extended to examination of suspected samples of hair, etc.

It would be advisable to require the Registrar to communicate with the Coroner in all fatal cases of anthrax.

When possible, walls and pavements of Factories and Workshops should be painted or glazed so as to be easy to clean and disinfect.

In the treatment of human anthrax a great advance has been made by the introduction of Sclavo's serum. It has been pointed out that at present in this country the serum has not achieved the success that it undoubtedly has in Italy, but that there are several reasons which will account for this, the chief being too late administration, and in too small doses. Therefore the necessity for an early diagnosis in all cases is apparent. Early diagnosis of anthrax being difficult, it is essential for a medical man to be attached to each factory, or group of factories, to whom all cases may be referred so that in making a diagnosis the nature of the employment may be taken into consideration: by this timely vigilance remedies, harmless in any case, may be used with far greater prospect of success.

The duties of certifying factory surgeons might be extended with advantage to include the above work, and that there may be no delay they should be supplied with serum by the Home Office. The surgeons should collect samples of suspected material for bacteriological examination, should undertake the entire treatment of all cases of anthrax, and, in conjunction with the local Factory Inspector, conduct an enquiry into the source of infection.

Employees absent from work should report to the employer the cause, and in the case of illness of any kind the employee should be visited at once by the certifying factory surgeon.

The employer should exclude as far as possible work-people with cuts or abrasions unless suitably covered, and for the carrying out of all regulations each factory and workshop should be supplied with, or compelled to supply, means for dressing small cuts, etc.

All cases of human anthrax whether industrial or agricultural should be notified. Both human and animal cases of anthrax should be notified to one authority, or to both the Board of Agriculture and the Home Office, so that if thought advisable the enquiry may be made in common.

Human anthrax being so closely associated with animal anthrax more systematic efforts should be made (1) by limiting the spread of the

disease in nature, and (2) by the immunisation of animals against anthrax, to exterminate the disease among animals.

As to the first, the *Bacillus anthracis* rarely if ever forms spores in the body, and consequently, if the bacilli can be confined to the blood and tissues of carcasses of animals that have died from anthrax, in the course of putrefaction the anthrax bacilli die out very rapidly. Unfortunately before death the animal by its discharges sheds into the air myriads of bacilli, which rapidly spore and, given suitable conditions of temperature and soil, germinate, multiply, and spore again; hence it is necessary to dispose of the carcase without shedding of blood, so that no part may be used, either (1) by burning, or (2) by deep burial in quicklime; these methods are equally effective, but perhaps for smaller carcases burning is to be recommended, and for larger ones deep burial. All places likely to have been contaminated with any discharges should be thoroughly disinfected, as with 1 in 1,000 corrosive sublimate.

Regulations exist in most European countries as to disposal of carcases of animals that have died of anthrax, but there is some reason for believing that these regulations are not properly enforced in a great number of districts abroad.

Immunisation of animals against anthrax by injection of attenuated bacilli has been largely carried out. In France by Pasteur's method the loss of sheep from anthrax is said to have fallen from 10 to 1 per cent., and of oxen and cows from 5 to 33 per cent. Yet it is stated the figures are fallacious, many of the animals vaccinated not being exposed to infection; and official returns by Cope indicate that the mortality remains as high as ever; further, while Pasteur's first vaccine is mild and harmless yet the second vaccine is dangerous and often fatal, sheep being more liable than cattle; besides, the animals still contract anthrax through the intestines, the common mode of infection, and the time of so-called immunity is not known.

Since Pasteur's experiments the most successful attempts have been made by Sclavo (1903) and Sobernheim (1904).

Sclavo (1903) injected into asses attenuated cultures of anthrax bacilli first, followed by virulent ones, until a high degree of immunity was obtained. The serum of the ass so immunised was found to have strongly protective and curative properties, and, as has been shown, is largely used in cases of human anthrax.

Sobernheim independently elaborated an almost identical serum which is largely used for the protective inoculation of cattle. The serum

is injected into one side of the neck or thigh, and the culture, Pasteur's Deuxième Vaccine (continued growth of anthrax bacilli for 12 days at 42 to 43° C.) into the other side. This method is now widely used in Germany and Brazil and it is said with good results.

Investigations should be undertaken in each country or by some international organisation, to determine accurately the nature and extent of anthrax districts, which should be then kept under supervision and, where possible, drained or rendered innocuous by other means. Such measures would result in a considerable reduction in anthrax among animals and consequently among human beings. Such an organisation would give warning of the prevalence of anthrax in these districts, so that export of infected material might be controlled.

Refuse from factories where raw animal materials are used, certain manures, and imported food stuffs all form a frequent source of infection to animals and indirectly therefore to man.

Dust from horsehair factories is not infrequently sold to manure manufacturers, who after mixing with other ingredients sell it for spreading upon the soil. The profit in the case of one horsehair firm amounted to £150 per annum, so that manufacturers are not likely to forego such profits except under compulsion. Hence it is necessary to prevent the sale of dust arising in the manipulations of dangerous or non-disinfected raw animal products and to do this separate tables and rooms should be used for such material. Such dust should be burnt. The effluent from wool, hair, and skin factories should be rendered inert by some reliable process, such as prolonged boiling, before being discharged, or treated by a suitable strength of some such disinfectant as cyllin.

That imported food stuffs are also a source of infection is illustrated by the case of a man selling, and in part grinding, cake and fodder imported from Prussia and North Russia who developed anthrax.

Where practicable the subjection of the constituents of the various cakes, when in the moist or semi-liquid state, to a temperature of 212° F. for 5 minutes would remove much of the danger from this source. Indian or other meals containing anthrax spores in a dry state would require a much higher temperature to render them safe.

Other general measures as notification of all cases of deaths of animals from any acute disease and of those rendering necessary slaughter on the farm are desirable. A fee should be paid for notification and compensation for animals slaughtered, while failure to comply with these regulations should be punishable by a heavy penalty.

Animals, except in emergencies, should not be slaughtered or their carcases disposed of except on licensed and inspected premises; and, in all cases of animals slaughtered otherwise than by butchers in the ordinary course of their business, a veterinary should inspect the carcase, and give a certificate of the cause of death or disease, stating the uses to which the carcase may be put. A copy of the certificate to be forwarded to the Board of Agriculture as well as to the Medical Officer of Health.

Information should be furnished to Factory and Market Officials; no butcher or knacker should purchase the carcase without having seen the certificate.

#### CONCLUSIONS.

1. Among workers in horsehair and bristles in this country, malignant pustule is by far the most common form of anthrax; other forms occur occasionally.

2. Anthrax in the human subject, whether of industrial or agricultural origin, is derived entirely from animals affected with the same disease. The prevalence of human industrial anthrax varies with the greater or less quantity of material from infected districts which is used. This depends on the state of trade, while the prevalence of agricultural anthrax varies directly with the amount of animal anthrax.

3. The percentage of fatal cases among bristle and horsehair workers is slightly less than that in any other industry, and compares favourably with mortality from anthrax in similar industries in France and Germany; of these workers the disease is more fatal to women than to men.

4. In this country industrial anthrax is somewhat more common than that arising from agricultural pursuits, 55% of deaths arising under industrial conditions, and 12% of deaths arising in manipulation of horsehair and bristles.

5. Anthrax forms a professional risk to horsehair workers of 296% and to brushmakers of 029%. These figures compare favourably with those of similar industries in France and Germany.

6. In horsehair manipulations the risk to male workers is five times as great as the risk to female workers. In brushmaking the risk to males is twice that to females.

7. The risk of infection from horsehair is more than eight times that from bristles.

8. Bristles are not so apt to give rise to infection as horsehair because they are in a further state of preparation and have passed through more hands before importation. For the same reason tail hair is less likely to give rise to infection than mane hair.

9. Infection may arise in any of the horsehair processes, but is more common in the earlier ones and in the manipulation of short hair.

10. There is some small risk of infection from all kinds of bristles and horsehair, including English horsehair; but by far the most risk attaches to Chinese, Russian and Siberian horsehair. The risk of infection from bristle riflings is not great, as probably little of the dirt is animal in origin.

11. Malignant pustule is most common on the exposed and least frequently washed parts of the body, as the face and neck. The nails which harbour dust containing spores are a chief source of infection.

12. The mortality varies with the position of the pustule owing to the difficulty of diagnosis and treatment in certain parts.

13. Infection is not infrequent by conveyance of spores in clothes, finger nails etc. to non-workers.

14. Anthrax is endemic among animals all over the world. It is more prevalent in most countries in the hot summer months and in certain places.

15. The amount of actually infected material imported into this country is probably not large compared with the total bulk.

16. There are present both in bristles and horsehair three types of bacilli very closely resembling the anthrax bacillus but no one of them is pathogenic. The bacillus of malignant oedema is also commonly present.

17. Anthrax in the human subject being derived entirely from animals, it follows that to dispose of the disease from man, it is necessary to make greater efforts to do away with the disease among animals on the lines indicated.

18. Central disinfection is possible in the case of horsehair, but it would probably meet with much opposition from the trade and is not to be recommended.

19. Steam disinfection if carefully carried out, the steam not being so damp as to injure the material, or too dry to be efficacious as a germicide, or at a pressure not above 4 or 5 lbs. to the sq. inch, greatly diminishes the risk, but is not certainly effective in destroying all spores.

20. Immersion of raw material in liquid disinfectants is at present the best method of disinfection. Cyllin (1 in 100) at a temperature not higher than 120° F. is the most suitable though not absolutely effective. The gumming of the hair together is said to be obviated by the addition of a little alkali.

21. An extension of the duties of certifying factory surgeons is advisable, so that early and efficient treatment may be undertaken which is the only method of reducing the mortality.

22. In malignant pustules use of the cautery and subcutaneous injection of serum is advisable in all cases. In internal anthrax injection into the veins is necessary at the earliest moment.

23. Of other regulations the use of washing with nail brush in soap and water with the addition of cyllin or other suitable disinfectant, use of overalls, gloves and respirators etc., are very important.

It is of the utmost importance that every effort should be made to abate or abolish the ravages of anthrax because of its insidious nature, its fatal character, and its widespread occurrence.

## VARIATION IN SUSCEPTIBILITY OF GUINEA-PIGS TO DIPHTHERIA TOXIN.

By H. J. SÜDMERSEN, PH.D., AND A. T. GLENNY, B.Sc.

(*From the Wellcome Physiological Research Laboratories,  
Brockwell Hall, Herne Hill, London, S.E.*)

### INTRODUCTION.

DURING the course of investigations which have been made during the past two years on the transmission of immunity to diphtheria toxin in guinea-pigs it was observed that a seasonal variation in the dose was necessary in order to cause the death of control animals. This variation was seen to follow somewhat the time of year at which the tests were made, so that during summer a larger dose was necessary than during the winter. We found also that in winter a guinea-pig took a longer time to come up to the standard weight of 250 grms. than in summer, and that the lethal dose varied directly with its age.

So far as we are aware there are no records at present in the literature of such variation in the case of diphtheria intoxication. Reid Hunt and Seidell have found that the lethal dose of acetonitril to mice is lower during the summer than during the winter months; the reverse of that which we have observed in the case of diphtheria toxin on guinea-pigs. In their paper no mention is made either of the ages of the mice used or of the variation if any in susceptibility dependent upon age apart from weight.

### METHOD.

The toxin selected was a stable one, having been prepared in December 1900. On each occasion of testing guinea-pigs, the progeny of treated parents which had arrived at the standard weight of 250 grms., control tests were made on normal guinea-pigs of the same weight in order to guard against false conclusions arising from any unexpected deterioration. In this way results have accumulated from more than 100 normal guinea-pigs. As will readily be understood a large number of animals were not necessary as controls on each occasion, the tests

*Diphtheria Toxin*

being repeated every few days. The results obtained therefore cannot be taken as giving exact quantitative differences between the minimal lethal doses but rather as indications of the extent of the differences. To obtain more accurate determinations it would be necessary to inject long series of guinea-pigs and definitely fix the lethal dose for each season of the year. This we are now carrying out.

## RESULTS.

*Variation in Resistance.*

The minimal lethal dose of toxin J90A which was used throughout these investigations is here given for different times from Jan. 1908 to July 1909. It is seen that in addition to the annual variation, there is an apparent increase in toxicity during this present year as compared with the previous. This we suggest may be due to differences in climatic conditions of the two years.

TABLE I.

*Showing variation in the approximate m.l.d. of diphtheria toxin for guinea-pigs according to season.*

Month												Fatal dose
January 1908	...	...	...	...	...	...	...	...	...	...	...	0·008 c.c.
May 1908	...	...	...	...	...	...	...	...	...	...	...	0·009
September 1908	...	...	...	...	...	...	...	...	...	...	...	0·010
November 1908	...	...	...	...	...	...	...	...	...	...	...	0·0075
January 1909	...	...	...	...	...	...	...	...	...	...	...	0·006
April 1909	...	...	...	...	...	...	...	...	...	...	...	0·0065
July 1909	...	...	...	...	...	...	...	...	...	...	...	0·009

These numbers when plotted out give the following curve.

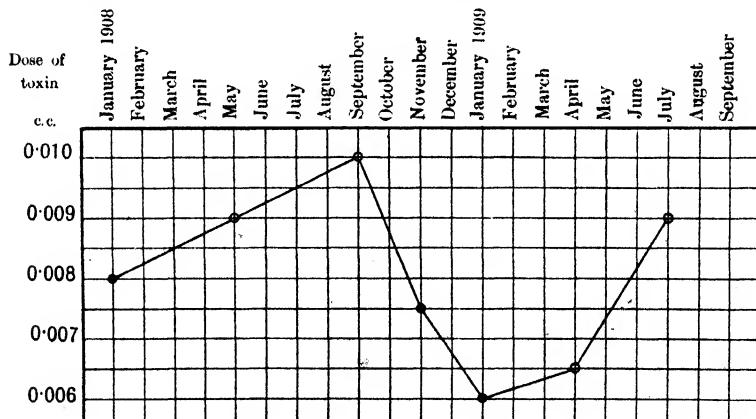


Chart I.

These figures as above stated are to be considered only as close approximations to the exact lethal doses. The most accurate indications are given by taking the percentage number of animals that survive beyond the 5th day when injected with a certain dose. For the purpose of Table II the results are divided into three sections according to the results at three different doses—0·006 c.c., 0·008 c.c. and 0·010 c.c. In forming the table any survivals at a higher dose are included among those at a lower dose on the assumption that a pig that survived a higher dose would naturally have survived a lower dose had that dose been given instead. In the same way any deaths at a low dose are included among the deaths at a high dose.

TABLE II.

*Showing number and percentages of survivals and deaths of guinea-pigs injected with certain doses of diphtheria toxin at different seasons.*

Months	Dose :- 0·006 c.c.		0·008 c.c.		0·010 c.c.		Percentage survivals at		
	Deaths	Survivals	Deaths	Survivals	Deaths	Survivals	0·006 c.c.	0·008 c.c.	0·010 c.c.
Jan. 1908	0	4	5	0	—	—	100	0	—
Feb.	—	—	1	2	—	—	—	66	—
Mar.	—	—	1	0	—	—	—	—	—
April	—	—	—	—	8	0	—	—	0
May	—	—	1	6	11	0	—	85	0
June	—	—	0	5	1	4	—	100	80
July	—	—	—	—	0	3	—	—	100
Aug.	—	—	—	—	0	1	—	—	—
Sept.	—	—	0	3	4	1	—	100	20
Oct.	—	—	8	1	13	0	—	11	0
Nov.	2	6	11	0	—	—	75	0	—
Dec.	5	2	8	0	—	—	28	0	—
Jan. 1909	11	0	—	—	—	—	0	—	—
Feb.	3	1	—	—	—	—	25	—	—
Mar.	4	2	—	—	—	—	33	—	—
April	1	6	3	0	—	—	85	0	—
May	—	—	3	3	12	0	—	50	0
June	0	4	11	3	—	—	100	21	—
July	0	4	13	3	14	0	100	18	0
Aug.	0	10	12	7	20	1	100	36	4
Sept.	—	—	5	0	—	—	—	0	—

These results are expressed in graphic form in Chart II. This consists of three curves—the lowest depicting the percentage number of survivals at 0·006, the middle curve at 0·008 and the top curve at 0·010 c.c.

*Diphtheria Toxin*

Within the limits of a third to sixth day death the product of toxin dose and lethal time appears to be a constant when the average of a number of results is taken (Dean and Craw, *Journ. of Hygiene*, 1907). Table III shows that the value of this product varies with the time of year.

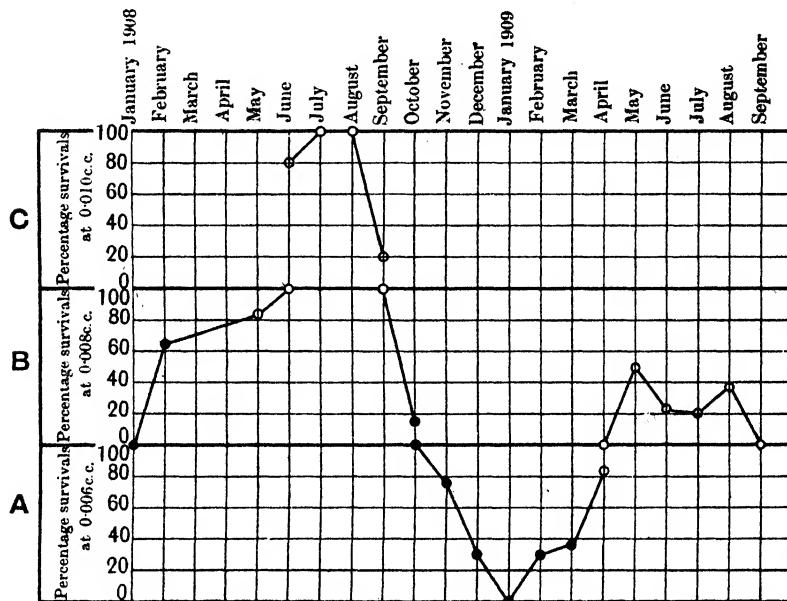


Chart II.

TABLE III.

*Showing that the product of lethal dose and lethal time varies according to the season.*

Date	Product of Toxin dose (in c.c.) and lethal time (in days)	No. of observations
January—March 1908	.0249	9
April—June ,,	.0496	28
July—September ,,	.0460	32
October—December ,,	.0323	29
January—March 1909	.0284	15
April—June ,,	.0403	20
July—September ,,	.0444	22

These numbers when plotted out give the following curve.

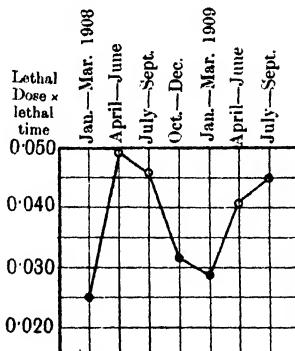


Chart III.

#### *Rate of Growth.*

It was at first thought that the variations in age of guinea-pigs of 250 grams weight in summer and in winter might account for the difference in susceptibility. At different times a number of animals

TABLE IV.

*Showing average ages of 250 gram weight guinea-pigs at different seasons of the year.*

Date	No. of animals	Average age
October—December 1908	71	45.2 days
January—March 1909	71	44.0 „
April—June 1909	57	34.6 „
July—September 1909	33	29.8 „

These numbers when plotted out give the following curve.

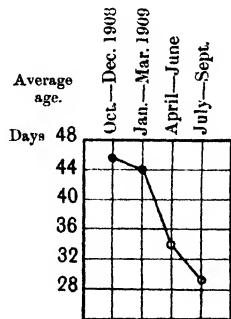


Chart IV.

*Diphtheria Toxin*

were put aside and the times taken to reach 250 grams weight were noted. From these records it has been calculated that the average age of 250 gram guinea-pigs in the six months of winter (Oct.—March) is 44·6 days compared with 32·8 days in summer. Individual variations are so great compared with the number of cases taken that it is necessary to divide the results obtained quarterly and not monthly. This has been done in Table IV and Curve IV.

From the tables and curves so far recorded it appears that in summer larger doses are required to kill a pig of 250 grams than in winter and also that in summer the age of 250 gram pigs is much less than in winter. A few experiments were carried out to determine whether the variation in susceptibility depended upon the age of the guinea-pigs or whether both were dependent upon some third factor. In May, June and July 1909 pigs of known age were whenever possible used as control pigs and the results are all recorded in Table V.

TABLE V.

*Showing that susceptibility of guinea-pigs to diphtheria toxin varies inversely as the age of the animal.*

Date of experiment	Age of guinea-pig in days	Dose of toxin injected, in c.c.	Number of days till death
3 May 1909	41	0·008	4
	49	„	7
10 May	27	0·009	5½
	38	„	4
18 May	46	0·009	4
	57	„	5
21 May	43	0·009	3
	49	„	3
25 May	20	0·008	3
	28	„	3
	35	0·009	4
15 June	31	0·008	3
	31	„	3
25 June	27	0·008	3
	43	„	11
18 July	26	0·008	4
	32	„	6
20 July	29	0·008	4
	35	„	6
	68	0·009	4

From a consideration of this table it appears in general that the fatal dose for an old guinea-pig is greater than that for a young guinea-pig of the same weight. A single exception (for which we can suggest no explanation) is seen in the case of two guinea-pigs injected on May 10th. In one case where no difference in date of death is noted the two pigs are of the same age. In the other two cases the difference in age is very small and it is quite possible that a difference would have been observed had observations of death been more frequently taken than morning and evening. If, then, age were the only factor causing variation in susceptibility, it would follow that the fatal dose of a toxin would be higher in winter than in summer, but the reverse is the case.

#### *Relationship of Loss in Weight to Time of Death.*

The loss in weight of guinea-pigs in the first few days after an injection may often be taken as an indication of the day upon which the animal will die. The ratio between the loss in weight and day of death varies with the time of year.

TABLE VI.

*Showing the loss in weight in grams during the first 24 hours of guinea-pigs injected with diphtheria toxin which died on either the fourth or fifth day after injection.*

Time of year	No. of experiments	Loss of weight
April—June 1908	27	11·1 gms.
July—Sept. ,,	19	8·1 ,,
Oct.—Dec. ,,	56	13·2 ,,
Jan.—Mar. 1909	47	14·9 ,,
April—June ,,	78	5·1 ,,
July—Sept. ,,	138	4·0 ,,

These numbers when plotted out give the following curve.

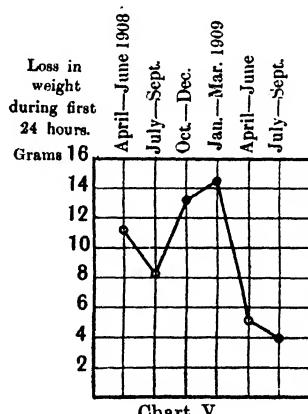


Chart V.

*Diphtheria Toxin*

TABLE VII.

*Showing the loss in weight in grams during the first 24 hours of guinea-pigs injected with diphtheria toxin, which died between the 6th and 10th day.*

Time of year	No. of experiments	Loss of weight
April—June 1908	13	6·1 gms.
July—Sept. ,,	9	5·5 ,,
Oct.—Dec. ,,	11	11·8 ,,
Jan.—Mar. 1909	25	10·8 ,,
April—June ,,	144	5·5 ,,
July—Sept. ,,	61	2·8 ,,

These numbers when plotted out give the following curve.

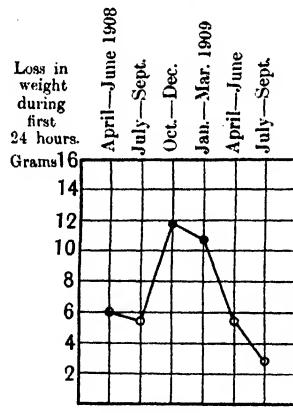


Chart VI.

The comparison between the summer months from April to September and the winter months from October to March is shown in Table VIII.

TABLE VIII.

	4—5th day death	6—10th day death
Summer	5·3 gms.	4·4 gms.
Winter	14·0 ,,	11·1 ,,

For the purpose of comparison a curve is shown on Chart VII for the average of the daily temperature for each month as given in the official reports of the Meteorological Office during the period covered by the results.

If we compare the number of months when the average temperature was below  $40^{\circ}$  F. during the winter of 1907—8 with that of 1908—9, and also the number of months when the average temperature in summer was above  $56^{\circ}$  F. or  $60^{\circ}$  F., it is obvious that distinctly more wintry conditions have prevailed during this latter season. This may

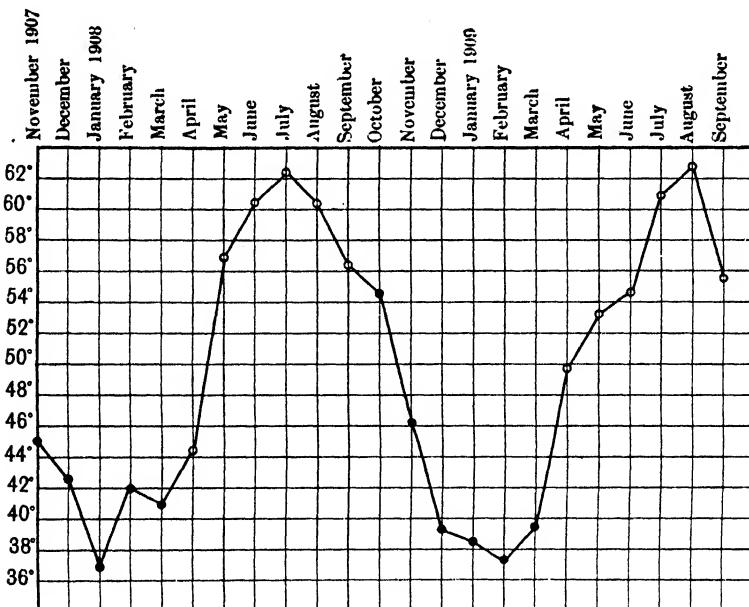


Chart VII. Curve showing average monthly temperatures.

help to explain the fact above recorded and shown in Curve No. 1 that our guinea-pigs have exhibited a greater susceptibility to diphtheria toxin during the present year as compared with last year. It should be pointed out that the guinea-pigs used in the experiments were kept in a special animal house artificially heated in winter.

#### SUMMARY.

1. A larger dose of toxin is necessary to kill a guinea-pig of the same weight in summer than in winter.
2. The rate of growth of guinea-pigs is more rapid in the summer months.

3. The weights of guinea-pigs are least affected by lethal doses in summer.
4. For guinea-pigs of the same weight the fatal dose increases with the age.

#### CONCLUSION.

These results show that it is of importance when dealing with the fatal dose of a toxin or with any conclusion drawn from the loss in weight of an animal, to take into consideration the time of year when the experiment was performed.

## ON THE PRODUCTION OF ANTITOXIN BY THE INJECTION OF FILTRATES OF CULTURES OF NON-VIRULENT DIPHTHERIA BACILLI.

By J. A. ARKWRIGHT, M.D.

(*From the Lister Institute of Preventive Medicine.*)

WE are still in doubt regarding the exact position of those bacilli which, morphologically and culturally, appear to be identical with the Klebs-Loeffler bacillus but which differ from it only in the absence of specific pathogenic power for laboratory animals. Most writers regard such non-virulent strains as true diphtheria bacilli which have lost their virulence and toxigenic power either temporarily or permanently. Others however are inclined to place them in a group allied to that of the genuine Klebs-Loeffler bacillus and express doubts as to whether they at any time possessed a claim to pathological significance (Graham-Smith, 1904).

The experiments detailed in this brief communication were made in the course of an attempt to demonstrate the presence of small quantities of specific diphtheria-toxin in broth culture filtrates of these non-virulent strains.

Three non-virulent strains were employed. They were all isolated from the fauces during an investigation of a diphtheria outbreak in a boys' school. Details of the bacteriological findings in connexion with this epidemic have been put on record by the writer (Arkwright, 1908).

Notes regarding the strains employed:

Strain "A" was recovered from the throat of a boy, who two months before had a sore throat and at the time of examination still had a nasal discharge. This strain was of the medium-long type.

Strain "B" was an extremely long and segmented form, while strain "C" was a rather short type. These two latter strains were isolated from boys who had not been observed to suffer from sore throat.

In glucose-peptone water all three strains produced an acid reaction, but cane-sugar media were not altered.

The subcutaneous inoculation of large doses (e.g. 5 c.c.) of 2-day and 8-day old broth cultures, produced no illness in guinea-pigs and, when massive doses large enough to kill were given intraperitoneally, no protection or postponement of death was afforded by the preliminary or simultaneous injection of antitoxin.

Although all attempts to demonstrate by direct methods the presence of toxin in broth culture filtrates of these strains had failed, it was thought that the injection of these filtrates might lead to the development of antitoxin in the horse, even if only very minute quantities of toxin or toxoid were present. Similar experiments by Petrie (1905) were made with strains of Hofmann's bacillus, but with negative results.

#### *Scheme of Inoculation and Serum-tests.*

Before the commencement of the inoculations the horse was bled on 4th November 1907 and the normal antitoxin-content of its serum estimated. It was found to possess a quarter of a unit per cub. cent.

Filtrates of 9-day broth cultures of strain "A" were then inoculated in the following quantities at intervals of two or three days: 8th Nov. 1907, 0·5 c.c.; 11th Nov., 1 c.c.; 13th Nov., 5 c.c.; 15th Nov., 10 c.c.; 18th Nov., 30 c.c.; 20th Nov., 80 c.c.; 22nd Nov., 200 c.c.; 25th Nov., 400 c.c.; 27th Nov., 1000 c.c. On Dec. 7th (i.e. 10 days after the last injection) the horse was bled to the amount of six litres.

The serum was now found to contain 4 units per cub. cent.

After a rest, the horse received two large doses of filtrate from strain "B", viz. 24th Jan. 1908, 200 c.c.; and 27th Jan., 600 c.c.

On 11th Feb. 1908 it was again bled when the serum showed an antitoxin value of 25—30 units per cub. cent.

During March 1908 the horse received further injections of filtrate from strain "C" but no appreciable rise in potency resulted.

#### CONCLUSIONS.

(a) Filtered broth cultures of two strains, which were morphologically and culturally indistinguishable from virulent Klebs-Loeffler bacilli but which possessed no pathogenicity for guinea-pigs and apparently were non-toxigenic, led to the development of antitoxin when injected in large quantities into a horse.

One strain raised the potency of the serum from  $\frac{1}{4}$ th unit (possessed normally) to 4 units per cub. cent. Further treatment with a second strain caused a further rise to 25 units per cub. cent. Subsequent treatment with a third strain produced no further elevation of the antitoxin-content.

(b) These strains though completely non-virulent must be regarded as true *B. diphtheriae* in virtue of their power to excite the production of antitoxin in the horse.

(c) Whether these strains have lost their pathogenic properties permanently or only temporarily cannot be stated, but all attempts to raise their virulence or toxicogenic power by guinea-pig passage or by cultural methods have so far failed.

Further experiments are in progress on the antitoxin-producing power of various non-virulent strains of *B. diphtheriae*.

To Dr MacConkey I am much indebted for superintending the inoculation of the horse at Elstree.

#### REFERENCES.

- ARKWRIGHT, J. A. (1908). Observations on the Bacteriology of an Epidemic of Diphtheria in a School. *Journ. of Hygiene*, Vol. VIII. p. 48.  
GRAHAM-SMITH, G. S. (1904). A study of the virulence of Diphtheria bacilli isolated from 113 persons, etc. *Journ. of Hygiene*, Vol. IV. p. 258.  
PETRIE, G. F. (1905). On the relationship of the Pseudo-diphtheria to the Diphtheria bacillus. *Journ. of Hygiene*, Vol. V. p. 134.

**ROUTINE METHODS OF SHELLFISH EXAMINATION  
WITH REFERENCE TO SEWAGE POLLUTION.**

By JAMES JOHNSTONE, B.Sc.

(Fisheries Laboratory, University of Liverpool.)

**CONTENTS.**

	<b>PAGE</b>
Introduction . . . . .	412
Legal powers of fishery authorities . . . . .	413
Methods of sampling . . . . .	416
Isolation of intestinal bacteria . . . . .	421
Precision of bacterial counts . . . . .	423
Characters of organisms isolated . . . . .	424
Cultural reactions of <i>B. coli</i> and related organisms . . . . .	426
Variability of reaction . . . . .	431
Standards of permissible impurity . . . . .	434
Cleansing of polluted shellfish . . . . .	436
Summary and conclusions . . . . .	438

IT is unnecessary to introduce the observations which I propose to make by any reference to the gravity of the problem of the sewage contamination of edible shellfish, in its relation to the public health: this side of the question is already familiar to readers of the *Journal of Hygiene*. But the fact that some forms of disease are conveyed—perhaps perpetuated to some extent—by the consumption of such articles of food is one which is now of the greatest possible importance to those engaged in the administration of the sea fisheries; and this aspect of the question is in some danger of being ignored. About half a dozen years ago the Royal Commission on Sewage Disposal invited evidence from representatives of the Fishery Authorities, and it was then foreseen that the question was likely to become one of very great practical importance; and some of the Fishery Committees began to accumulate information with regard to the pollution of the layings under their control.

The Lancashire and Western Sea Fisheries Committee which has jurisdiction over the largest shellfish producing areas in the British Isles began to examine into the condition of the shellfish beds about 1904, and since then a great amount of information has been obtained. Every natural bed and laying has been surveyed and charted; every sewer outfall has been examined with respect to its influence on adjacent layings; while systematic bacteriological analyses have been carried on ever since. It was soon seen that the condition of some of the shellfish beds constituted a most serious menace to the public health; but it was also discovered that the Fishery Authorities had no power to prevent the marketing of even such dangerously polluted molluscs. Repeated representations were made to successive Ministers for legislation designed to confer this power on some authority; but so far these representations have been unsuccessful. During the last year particularly the danger foreseen in 1904 has been realised and the industry is now suffering from the effects of periodic "scares."

This in itself is sufficient reason for legislation. It is also unfortunate that the Fishery Committees are placed in the difficult position of being expected by the Health Authorities to take steps to prevent the export of polluted shellfish. There is no coordination between the two sets of Committees, and the Public Health Officers are not usually aware of the legal incapacity of the Fishery Authorities to stop the evil<sup>1</sup>. It has happened, in the proceedings of Public Authorities, that the blame of the distribution of disease by means of sewage-contaminated mussels has practically been laid on the Fishery Committees: the fact is, of course, that the latter are absolutely powerless to deal with the matter.

In England and Wales the control of the shellfisheries lies entirely with the Fishery Committees, except where exclusive rights of fishing belong to some person or corporation. With respect to public fisheries the Committees have power to prohibit entirely, or restrict in any manner desirable, the methods, or seasons of taking mussels, cockles, or other shellfish. Thus there are "close seasons"; illegal methods and instruments of fishing; and size limits below which shellfish may not be taken. The Committees have power to prohibit the discharge into the sea of any substance "detrimental to sea-fish or sea-fishing"; and they

<sup>1</sup> There is the less reason for this since a particularly clear statement of the law with regard to the pollution of tidal waters is contained in the 4th Report of the Royal Commission on Sewage Disposal (1904); while the Proceedings of the Fishery Committees are accessible to public officers who wish to consult them.

have power to spend public money in stocking or restocking shellfisheries; in transplanting these animals; in artificial cultivation; and in making scientific experiments for these purposes.

Apparently these powers might be used so as practically to prevent shellfish which are undesirably polluted from reaching the public markets: in practice they are entirely useless. Thus a Fishery Committee may close against fishing any part of the sea, or foreshore, within territorial limits, from which shellfish are being taken, *but this closure must be in the interest of the fishing industry.* A byelaw prescribing a regulation can only be suggested by the local fishery authority: it is enacted by the central authority, in this case the Board of Agriculture and Fisheries. Before confirming the byelaw the Board enquire into the reasons alleged for the institution of the regulation. A Fishery Committee may obtain a byelaw in the interest of the industry, but not in that of the public health. In 1904 the Lancashire Committee sought power to enforce a regulation prohibiting the taking of mussels from a certain part of the shore within the area under their jurisdiction. The mussel bed in question had long possessed an evil reputation. Several large sewer outfalls opened almost directly on to it, so that the shore on which the mussels were growing was grossly polluted by faecal matter. There was direct epidemiological evidence of the transmission of disease by means of these shellfish; and the results of bacteriological analyses were most unequivocal. The local Medical Officer of Health, recognising the dangers of the laying, obtained permission from his Committee to exhibit notices enjoining the fishermen not to take the mussels, but this prohibition could not, of course, be enforced. Finally the Fishery Committee drafted a byelaw closing the fishery, and submitted this to the Board of Agriculture and Fisheries for approval. The Board refused to confirm the byelaw, pointing out that the powers possessed by a local Fisheries Committee "did not extend to the making of byelaws for the closure of a mussel bed or other fishery for shellfish for the purpose of protection of public health." The mussel bed therefore still remains as an occasional focus of infection, for the Fishery Committee, which has power to close it, cannot do so in the interest of the public health; while the local Health Committee which can act for the protection of the public health cannot close a mussel bed.

There are two important limitations of the powers nominally enjoyed by the Fishery Committees with reference to the discharge of objectionable substances into the sea. If the discharge is sewage, and if it is

made by a local sanitary authority in virtue of power conferred on it by a local or general Act of Parliament, or by a Provisional Order confirmed by Parliament, the Section of the Sea-Fisheries Regulation Act (2, e) of 1888 which would otherwise enable the Committee to prohibit the discharge is nullified. Now it is apparently the case that the Public Health Acts have been regarded as conferring such powers upon the local health authorities. The latter are therefore enabled to discharge their sewage at any convenient spot into the sea, with, or without, regard to the situation of any local shellfish beds. It is true that the Local Government Board now takes steps to consult the Fishery Committees when such new sewer outfalls are being planned; and proper attention is paid to the question of the possible fouling of shellfish beds before the Board sanction the proposed works. But it is still the case that the majority of sewer outfalls have been planned in the past without sufficient regard for the shellfisheries. The second limitation is an even more serious one. The discharge must be "detrimental to sea-fish"—the latter term includes shellfish. Therefore in attempting to restrain a sanitary authority from discharging sewage in the neighbourhood of a shellfish bed the Fishery Committee would have to prove that sewage is detrimental to the molluscs. Now this is impossible, for the greater the amount—up to a certain high limit—of sewage reaching mussels, the better do the latter grow. We find therefore that mussels are always situated in such places where they receive drainage from the land. It is therefore impossible to prohibit the discharge of sewage near a shellfish bed on the score that injury results to the animals. The position of affairs was put very concisely by Lord Onslow—then President of the Board of Agriculture and Fisheries—at a meeting of representatives of Sea-Fisheries Authorities held in London in 1904: "If," he said, "anything is done to threaten the valuable life of the mussel I can step in and take the necessary steps to protect it, but if the mussel, in the enjoyment of crude sewage, should threaten the life of a human being I am absolutely powerless to interfere in the matter."

Neither do the Rivers Pollution Prevention Acts afford a remedy, for tidal waters—in which shellfish beds are situated—are excluded from the operation of the Acts. It is true that the Local Government Board may make an Order declaring a tidal water to be a "stream" within the meaning of the Acts, but this power has been reluctantly used.

Finally the powers possessed by the Fishery Committees under the Shellfish Act of 1895 vanish whenever it is attempted to utilise them so as to provide against the evil of polluted mussels or other shell-

fish reaching the public markets. Under this Act the Committees may spend public money in transplanting shellfish from places where they grow badly to places where they would grow well; or they may store shellfish so as to provide spawning reserves; or they may replenish an exhausted fishery. But they may not remove mussels from a polluted to an unpolluted area; nor may they provide storage ponds for the reception of the shellfish while undergoing a period of quarantine. To do these things would require the expenditure of public money "for the purpose of the protection of public health." And the fear of a surcharge by the Local Government Board Auditor weighs heavily upon the Local Fishery Committees.

Thus the position of the Committees is one of detachment with regard to the general question of the dissemination of disease by means of polluted molluscs. It is true that a good deal of local investigation has been carried out with the object of ascertaining the bearings of the question on the industry; but in regard to active measures for the safeguarding of the public the Committees, who might be expected to know most about the whole thing, are in a position of absolute legal impotence—an *impasse* produced by the lack of coordination between the two series of authorities. It appears to me that with the great exceptions of the work of Dr A. C. Houston (1904, *a*) and of Dr H. T. Bulstrode (1895), both of whom have investigated the question with regard to the sea-fisheries, the public health authorities have generally treated the question rather apart from the natural conditions under which shellfish are produced, and the practicability of taking steps to deal with polluted shellfish without necessarily interfering unduly with the fishing industry. So far as my own experience goes the Public Health Committees have not sought assistance from the fishery authorities, and have not paid attention—to the extent that is desirable—to the important interests involved: those of the livelihood of the shell-fishermen.

From this point of view therefore—that the general question is one that affects the fishery, just as much as it does the public health—it would seem useful to give some account of the experience gained in the investigation of the conditions as they obtain on the west coast of England and Wales.

#### **Methods of Sampling.**

A shellfish bed is usually a fairly considerable area. In the case of one west coast mussel fishery the total productive area is about 550 acres. Not all of this sea-bottom is fished at one time, for circumstances

usually dictate that the fishing is carried on at some one part of the whole district, and then after a period is shifted to some other part. In the district to which I refer a sub-area of about 25 acres is often the scene of a busy fishery. If the conditions with regard to pollution were uniform over all these 25 acres one sample taken from any spot would afford a reliable indication of the degree of pollution.

But the conditions are seldom so uniform. A mussel bed is usually a raised part of the sea-bottom—a "scar"; or it is the bottom and slopes of an estuary or channel; or perhaps the sides of a wall, or embankment. In most cases the shellfish are covered by water for a variable fraction of the whole twenty-four hours and are then laid bare by the tide. In most cases the shellfish at different parts of the same general area are exposed to varying degrees of pollution. An ordinary case is that of a channel of no great width, into which there discharge one or more sewer outfalls. Perhaps the engineer who designed the sewerage system provided for an intermittent discharge so that the effluent might be carried away by the ebb-tide; but it is usually safe to assume that the discharge is crude sewage; that it is continuous; and that the purification plant, if there is one, is not worked as efficiently as was contemplated in the designing. On the bottom and slopes of such a channel, and on one or more banks in the middle, are mussels. The tidal rise and fall is often considerable—it may be put at 15 to 25 feet, and it varies from day to day. At the time of high water of flood-tide the channel is filled with water which has come in from the open sea, and this is relatively, or perhaps practically, unpolluted.

All the time, during both flowing and ebbing tides, the sewers are discharging, unless the head of water due to the rise of the tide should bank back the effluent in the outfall pipes. But at the time of low tide the volume of water in the channel is minimal, and therefore the proportion of contained sewage is greatest then. Comparative cultures of similar volumes of water at different states of the tide give usually very different results. As a rule 1 c.c. of water from the channel at the time of high tide should be sterile to media demonstrating the existence of intestinal microbes only; while a similar volume of water taken when the tide is at its lowest will contain a significant number of such organisms: about 5 to 50 per c.c. is a likely range. In May 1908 I made such comparative cultures of samples of water taken from the Barrow Channel opposite to the Fisheries Laboratory. Two c.c. of water were taken every two hours and were plated in about twenty c.c. of neutral-red, bile salt, lactose agar. The results were as follows:

*Nos. of intestinal bacteria in 2 c.c. of water from Barrow Channel.*

Flood-tide water		Ebb-tide water	
5 hours before high water	0	6½ hours before low water	0
3    "    "    "	1	4½    "    "    "	0
1    "    "    "	0	2½    "    "    "	200
		½    "    "    "	+1000*

\* Counting was impossible because of the fusion of the very numerous colonies.

Thus the water at the time when the tide is lowest contains very many more intestinal organisms than when the tide is highest. The numbers of organisms contained in the unit volume—say one c.c.—varies with the proximity to the sewer outfalls. In 1906 I made a series of cultures of the water in the estuary of the river Conway. Samples of the flood-tide water were sterile (with regard to the particular medium mentioned above) but the numbers of organisms isolated from one c.c. were greatest at the upper extremity of the estuary, and least at the opening into the sea. The numbers varied from 0 to 77.

*Analyses of water from the estuary of the river Conway.*

(1 c.c. of water inoculated in neutral-red, bile salt, lactose agar, and incubated for 20 hours at 41·5° C.)

26 Oct. 1906. Low water.

Source of water	Nos. of intestinal bacteria per c.c.*		
1. Pools near high water mark on beach at mouth of estuary	0		
2. Mid-channel, near mouth of estuary ...	...	...	5
3. Mid-channel, higher up estuary ...	...	...	27
4. Mid-channel, opposite Conway ...	...	...	70

\* Averages of counts from two plates.

3 Dec. 1908. Low water.

Source of water	Nos. of intestinal bacteria per c.c.*	Physical condition of the water	
		Chlorine %	Salinity %
1. Mid-channel, in estuary just below Conway	38	5·81	10·52
2. Mid-channel, opposite Conway ...	36	1·88	3·42
3. Mid-channel, above Conway ...	20	0·58	0·95

\* Averages of counts from 4 plates.

Sometimes quite irregular results are obtained when an attempt is made to demonstrate the increase in the bacterial contents of the water of such an estuary with approach to the origin of the pollution, and

these are to be traced to unusual conditions of wind and tide causing eddies and surface drifts of water<sup>1</sup>.

Consider a part of a mussel scar which is exposed to the atmosphere for about two hours near low water of spring tides. As the tide ebbs it comes to contain an increasing proportion of sewage, and if the velocity of the stream is not too great the latter floats at the surface by reason of its lower density. Therefore just about the time when our hypothetical mussel bed is being laid bare by the tide it is being bathed in water in which the pollution is maximal; and in extreme cases the liquid flowing over it may be practically undiluted sewage. Now contrast the conditions with regard to risk of pollution obtaining on such a bed, with those encountered by mussels which are situated on a scar, or on the sides of a channel, so that they are only covered by the tide during two hours before and two hours after high water of neap tides. While the shellfish are being bathed with sea-water the pollution of the latter is minimal, and by the time that the ebbing tide has come to contain a significant proportion of sewage the mussels are laid bare and are no longer in contact with the polluted liquid.

Two such mussel beds may be so close together as to be known by the same name. Yet one might be highly polluted while the other might be so slightly contaminated as to be practically clean.

If there are mussels at the bottom of a relatively deep channel—say ten to twenty feet deep at low water of spring tides—and if sewers discharge into this channel between high and low water marks, it may nevertheless be the case that the shellfish are not so highly polluted as might be supposed. The sewage floats at the surface of the sea and may not come into contact with the shellfish until it is greatly diluted. But during high and strong spring tides the velocity of the stream may be sufficient to produce a mixture of the water which may bring about a greater degree of pollution. Also certain conditions of wind prevailing during the time of spring tides may cause the level of low water to be several feet lower than is normal, and this too may be a cause of increased pollution.

It is clear that much may depend on the precise conditions under which the sample is taken, and upon the precise spot. If this is so then

<sup>1</sup> It is necessary to bear in mind that such conditions may produce quite unexpected results. Thus a normally clean foreshore may become very foul for a short time as the result of drifts caused by unusual winds; and the same causes are likely to affect the drift of surface floats when these are employed to ascertain the direction that may be taken by a sewage effluent.

great caution is necessary in applying the results of analysis of a sample of shellfish purchased from a market stall or shop, *to the general locality from which the molluscs are said to have been taken.* Not only so but the Report must, in justice to the fisherman, consider the length of time which has elapsed since the shellfish were taken from the sea, and the conditions under which they have been stored. If water is taken from a well, or shellfish from the shore, for the purpose of analysis, care is usually taken to pack the samples in sterile vessels, and if inoculations cannot immediately be made the samples are usually stored in a refrigerator. What then is to be said of the interpretation of the results of the analysis of shellfish which may have been taken from the sea some six days before the date of the sampling, and which may have been stored in insanitary conditions in the meantime? The discovery of pathogenic organisms in such shellfish might indeed be conclusive proof of the origin of a disease or epidemic, but the tracing of the latter to the part of the sea from which the shellfish were alleged to have come might be erroneous. It is surely unfair to condemn a locality on the results of such an analysis made perhaps on moribund animals in which partial decomposition may already have begun.

The fullest possible information relating to the circumstances of collection of the sample, and the conditions under which the consignment of shellfish were stored *after removal from the fishery*, is absolutely essential. In one case which came within my own experience mussels were gathered from scars which, though not free from pollution, were still relatively free from significant contamination. But these scars were some distance from the nearest railway station, and the fishermen were obliged to bring them in their boats to a point on the beach near to the station, and to wash, sort, and pack them there. Occasionally the men were unable to send away the fish on the day of collection, and in these circumstances the bags containing them were stored overnight on the beach, where they were just covered with the tide. Unfortunately a sewer discharged a few yards away from the place where the shellfish were thus stored, and so the mussels, originally fairly clean, became effectually contaminated. It is not surprising therefore that a Medical Officer of Health, reporting on a sample of such mussels, said that they contained *Bacillus coli*, and that the bags, when opened, emitted an odour of sewage. But the interpretation which might have been put upon the results of this analysis—that the mussels generally which came from this locality were significantly polluted—would not have been justified.

It is therefore necessary to examine a number of samples from the same general locality in order to guard against the undue influence of special or accidental local conditions. If, for instance, there is a difference of ten feet in the level of a laying over which shellfish are situated care should certainly be taken to choose the molluscs from every part of the bed, and it is always desirable so to conduct the analysis that every one of the shellfish so sampled should be separately analysed.

#### Isolation of intestinal bacteria.

In all analyses made by myself the fish were examined on the day after that on which they were collected, and it was generally necessary to store them overnight in a refrigerator. All moribund molluscs (indicated by the gaping of the shells) were rejected. Isolation was always carried out by plating on the surface of the neutral-red, bile salt, lactose agar medium suggested by A. S. Grünbaum and E. H. Hume (1902). The mussels were washed under the tap and were opened so that the adductor muscles of the shell, the pedal muscles, and the muscles of the mantle, were alone cut through. The soft parts of the mollusc were retained in the right-hand valve of the shell. Sterilised knives were prepared, two for each shellfish. About 10 to 15 c.c. of the medium were previously poured into Petri capsules and allowed to set. Pipettes were made by drawing out quill glass tubing of about  $\frac{1}{4}$  inch in diameter and were sterilised, and a rubber teat attached to each. With care it is possible to make these little pipettes so that they may deliver approximately the same volume of liquid in each case. A slit was then made in the body of the animal immediately over the stomach, and through the dark-green "digestive gland"—really an extension of the lumen of the stomach—and the sample quantity of the stomach juices was withdrawn and placed on the centre of the plate, and evenly distributed over the surface of the latter by means of a wide platinum loop. The volume of fluid taken amounted to about 0·1 c.c. Usually a mussel contains enough to make two or three separate inoculations. It was found useful to dry the plates after inoculation by exposing them in the incubator with the lids slightly tilted up for about ten minutes. The colonies were counted after 20—24 hours' incubation.

Counts so made are only relative to each other and the absolute numbers of bacteria per shellfish cannot be deduced from them. It is

nevertheless probable that the majority of the contaminating organisms are contained in the cavities of the stomach and digestive gland. The method is inapplicable in the case of the cockle since the small size of the latter mollusc renders dissection very difficult. When cockles were examined, and when it was desired to make an estimate of the total numbers of bacteria per mollusc, either cockles or mussels, the method suggested by Dr A. C. Houston (1904, e) for oysters was adopted. Five mussels were opened so that the soft parts of each lay in one valve, and as much as possible of the fluid contents of these was poured into a small sterilised porcelain mortar: the water contained in the shell had also been drained into the mortar. The body of the mussel was then cut up into as fine pieces as possible with scissors and the pulp was poured into the mortar and rubbed up with the pestle so as to obtain as uniform an emulsion as possible. The emulsion was then put into a 250 c.c. CO<sub>2</sub> flask and the latter was filled up to the mark with sterile water. After mixing as thoroughly as possible one c.c. was plated by mixing with 10 c.c. neutral-red agar fluid at 39° C. A sample of cockles consisted of ten animals made up to 100 c.c. One c.c. of the mussel mixture contains 0·02, and one c.c. of the cockle mixture contains 0·1 animal. With such quantities there is seldom any difficulty in counting the colonies.

Three principal categories of colonies grow on such a plate: (1) Large rapidly growing red colonies varying in tint from deep crimson to pale pink. The deep colonies are lenticular in shape and grow in the direction of least resistance. They are usually surrounded by a slight opacity, or haze. (2) Small deep-red colonies. (3) Medium-sized colourless translucent surface colonies, often surrounded by a clear ring due to the discharge of the colour of the medium. Colonies of the first category are those regarded as produced by "intestinal bacteria."

Occasionally the liquid contained in the shell cavity was also examined, but I think that little is to be gained by this procedure. The pallial liquid is only the last portion of sea-water taken into the shell before the latter was closed. It is not likely that multiplication of bacteria takes place on the film of mucus covering the body of the animal, for the latter is ciliated and the mucus is rapidly removed and taken into the mouth. If there is an excess of bacteria in the water of the shell over that in the sea covering the laying this is probably due to the discharge of the excreta of the animal into the shell cavity after the latter has been closed.

**The precision of the counts.**

The numbers of colonies contained on the surface plates made by the first method may vary greatly, but this is due to the individual variation in the bacterial contents of the stomach fluid in different mussels; to the varying degree of concentration of the fluid; and to the error involved in the construction and use of the pipettes. It would be possible to make the latter strictly uniform in capacity but it is hardly worth while. If the numbers of colonies do not differ greatly it may be assumed that the conditions are nearly uniform over the area from which the sample was taken; but if they differ very much further sampling may be necessary. If, however, in the practice of the second method several plates be made from the same emulsion, using precisely the same volumes of liquid for inoculation, a somewhat large variation in the number of the resulting colonies may be observed; and this is due to errors of experiment. Thus five mussels were made up to 250 c.c. and one c.c. of the emulsion was plated in each of ten capsules. The emulsion had been very carefully made and the flask containing it had been allowed to stand until the heavier solids had settled to the bottom. The counts were: 210, 258, 274, 277, 302, 305, 352, 375, 453 and 730. One of these values, 730, is very great and may be rejected. If however the others be plotted they can be made to fit a normal curve of frequency error with modulus 96·14. The error of mean square is 67·97. The true value is therefore just as likely to be 244 or 380 as the average, which is 312. The probable error of the average itself is  $\pm 15\cdot58$ , and since the precision of an average varies with the square root of the number of observations a fairly large number of plates would have to be made to reduce the error greatly. Quantitative bacteriological analyses are often regarded as comparable in accuracy to the analogous modes of procedure employed by volumetric chemists, but it would appear that such methods are rude and inaccurate when compared with those of the chemists. I do not see how the method indicated in the last few paragraphs is to be greatly improved. The bacteria inhabiting the bodies of shellfish are mostly contained in the cavities of the alimentary canal and digestive gland and the juices of these cavities can only be set free by mechanical disintegration of the body of the animal. Sometimes there is comparatively little fluid in the alimentary canal, and it may be thick and viscid. If the whole of the soft parts of an

oyster or mussel were cut up and the liquid allowed to drain away it is probable that a variable amount would still adhere to the walls of the intestine, and in the tubules of the gland. An uniform emulsion can hardly be prepared; for the harder parts of the body, such as the muscle bundles, break up with great difficulty. These parts must be allowed to settle so as to bring the mixture into a form in which it can be manipulated with pipettes. The error in the analysis must therefore be considered and care should be taken that it is always less than the range of values which it is desired to bring into comparison.

#### **Characters of the organisms isolated.**

Organisms which divide rapidly when cultivated on neutral-red, bile salt, lactose agar; forming colonies after twenty to twenty-four hours' incubation at 42° C.; and which are about 1 to 2 mm. in diameter if they grow on the surface, or about  $\frac{1}{2}$  mm. in diameter if they grow in the deep, have been regarded as "colon-like" or "intestinal" bacteria. It seems probable that the majority of the organisms growing in this manner on the medium are such as find their normal habitat in the intestinal canals of man and the domesticated animals, but it is, of course, necessary to examine the truth of this postulate. If relative counts be made of the numbers of colonies produced as the result of the cultures of similar fractional parts of the bodies of shell-fish, or samples of sea-water, taken from localities known to present varying conditions as regards liability to pollution, it will generally be found that the less likely the chances of pollution, the fewer are the numbers of "colon-like" bacteria isolated by means of the medium in question. I have already referred to the comparative cultures of water from the Barrow Channel, and it seems to me that these prove the truth of the postulate. The water that comes in from the sea on the flood-tide must be regarded as practically free from pollution. It contains some colon bacilli, of course, but these are seldom present in one c.c. The tidal streams surge out and in from this channel so that twice in every twenty-four hours mixing and enormous dilution must take place. On the other hand the channel at the time of low water is comparatively narrow, and it receives the crude sewage of Barrow-in-Furness, Dalton, and some other communities. It must therefore contain an appreciable proportion of sewage. So also with the case of the Conway Estuary.

A comparison of the results of cultures of mussels and other shell-fish, taken from regions presenting very different conditions with regard to the possibility of pollution, leads to analogous conclusions. The worst-polluted mussels that have come into my hands were some taken from the shores of the Mersey Estuary near to the Egremont Landing Stage. A sewer discharged directly on the mussel bed, and four others were situated within a distance of about one mile, and in such positions that the first of the flood-tide must have carried the effluent almost on to the shellfish. The bed was very foul and accumulations of faecal matter, water-closet paper, and other debris were scattered over it. There was also direct evidence of the communication of disease by means of these particular shellfish. Ten mussels were examined and the numbers of colonies counted on ten plates, each inoculated with about 0·1 to 0·2 c.c. of the stomach contents, were 250, 250, 300, 600, 900, 1000, 1000, and in three plates counting was impossible because of the fusion together of the exceedingly numerous colonies.

In this case the topographical evidence showed that the pollution was gross, and the results of the bacteriological analysis were strictly concordant. Compare these results with those of an analysis of shell-fish brought in from the open sea. Mussels are not found at sea at a considerable distance from the land, but oysters are, and the latter may be used for the purposes of comparison. In 1904 I obtained two samples of the latter molluscs by dredging (A) near the Liverpool North-West Light Vessel, 12 miles from land, and (B) from the Morecambe Bay Light Vessel, about 16 miles from land. The sea at A is not entirely without the range of land pollution since the hoppers carrying dredged material deposit their loads in the neighbourhood; old boots, crockery, and similar refuse may be found when trawling thereabouts. The sea at B may be regarded as quite outside the reach of ordinary contaminating influences. Six oysters were taken from each locality, and a slit being made in the body over the stomach of each, about 0·25 c.c. of the stomach fluid was plated on the surface of the neutral-red agar. The same volume of fluid was also used for inoculations in previously boiled litmus milk, which was then heated to 75° C. for 20 minutes and incubated anaerobically. Four of the plates made were sterile and two gave each one colon-like colony. The organisms from one of these colonies did not, however, ferment lactose. All six oysters gave a typical *enteritidis* reaction. The oysters taken from locality B yielded sterile plates only, although about 0·5 c.c. of stomach liquid was used for the inoculations, and a period of 48 hours

was allowed for the incubation. Four of the six milk tubes were sterile after incubation while in the other two the milk was rendered acid, and a slight atypical clot was produced. This is the only analysis I have made in which all of the samples failed to give a typical *enteritidis* reaction. It has been pointed out that the value of such a negative result is very great and my experience confirms this statement<sup>1</sup>.

It seems probable then that the numbers of colonies growing on neutral-red, bile salt, lactose agar give reliable indications (in the majority of cases) of the grade of pollution. The reaction with this medium may therefore be regarded as a simple but satisfactory test for organisms of the *Bacillus coli* category. Nevertheless we are not absolved from the necessity of further examining the reactions of the organisms isolated.

#### Cultural reactions of organisms of the *Bacillus coli* group.

No pathologist is likely to have any difficulty in identifying organisms of the above group, but it is not an easy matter for anyone whose daily task is not bacteriological work to satisfy himself as to what small series of reactions he ought to apply, as a matter of routine, in the identification of *Bacillus coli*. This difficulty appears to me to be all the greater since those bacteriologists in this country who have had most experience in the examination of shellfish do not employ precisely the same series of reactions for the identification of *Bacillus coli*. The Table on p. 427 summarises the main tests employed by the bacteriologists who have had most experience of this work.

From these series of tests, and others which have been adopted by other workers, it should be possible to determine with certainty whether a particular organism is the typical *Bacillus coli communis*, or some closely allied form. But the large number of reactions which have to be applied render routine work difficult and tedious.

When I began this work I made use of the Table published by MacConkey (1901) for the identification of the organisms isolated from primary cultures in bile salt agar. The Table included the employment of glycerine, peptone, and litmus broth as a means of distinguishing *Bacillus coli* from some other nearly related bacteria, but it appeared later that there was some inconstancy in the reaction obtained with this medium;

<sup>1</sup> The experiments made by Dr Houston (1905) with regard to the occurrence of *Bacillus coli* in deep-sea oysters will be familiar to most readers. I have obtained similar results.

and it seemed that slight differences in the precise manner of preparation determined in some cases whether or not fermentation occurred. Further, it was necessary to wait for six days before it was certain that the broth would not ferment. Usually the majority of the tubes contained no gas after twenty-four hours incubation, but in about one-half the reaction occurred after four or five days. Although in most instances it is possible to determine whether or not an organism is motile, cases frequently arise where it is very uncertain that motility is not really exhibited. For these reasons both the glycerine fermentation and motility tests were abandoned. With regard to other cultural

Reactions employed	Workers			
	Houston (1904 a)	Klein (1905)	McWeeney (1904)	MacConkey (1901, 1906)
1. Formation of indole in peptone broth	+	+	+	+
2. Fluorescence in neutral-red broth ...	+	+	+	...
3. Acid and gas in lactose broth ...	+	+	+	+
4. Acid and gas in milk ...	...	+	+	...
5. Acid and gas in bile salt broth ...	...	+	...	+
6. Non-liquefaction of gelatine ...	+	+	+	+
7. Gas bubbles in gelatine "shake" cultures ...	...	...	+	...
8. Non-retention of Gram's stain ...	...	...	...	+
9. Growth in phenolated media ...	...	+	...	...
10. Acid and gas in glucose broth ...	...	...	...	+
11. Acid and gas in mannite broth ...	...	...	...	+
12. Acid and gas in dulcite broth ...	...	...	...	+
13. No reaction in inulin broth ...	...	...	...	+
14. No reaction in adonite broth ...	...	...	...	+
15. Voges' and Proskauer's reaction negative ...	...	...	...	+
16. Ratio of H <sub>2</sub> /CO <sub>2</sub> in glucose fermentations = 2/1 ...	...	...	...	+
17. Motility exhibited ...	...	...	...	+

The + signs indicate that the reaction in Col. 1 is employed.

reactions there is some doubt as to their applicability in the precise diagnosis of *Bacillus coli*. The formation of gas bubbles in gelatine appears to depend on the exact nature of the gelatine employed—perhaps on the purity of the other constituents of the medium. In one case about twenty tubes of this medium all failed to react, nevertheless the same organisms bubbled gelatine which had been kept for about ten months, and which had been prepared in the same laboratory and apparently from the same formula. The difference was possibly due to the greater concentration of the medium, for the jelly in each tube had shrunk up to the extent of about one fifth of the original

volume; but it was more probably due to some slight difference in the constitution of the medium. The formation of indole; the growth in phenolated media; the production of fluorescence in neutral-red broth; and the clotting and reddening of litmus milk, all appear to be general reactions which may be exhibited by organisms allied to the typical *Bacillus coli*. It would appear then that we are compelled to resort to fermentation reactions in pure sugars if we wish to identify the organisms

TABLE I.

*Reactions of 153 organisms isolated from Cultures on Neutral-Red, Bile Salt, Lactose Agar.*

	Bile salt broth	Glucose broth	Lactose broth	Mannite broth	Cane sugar broth	Litmus milk	Total	%
Coli-like organisms	ag	ag	ag	ag	0	ac	85	55
	ag	ag	ag	ag	ag	ac	36	23
Not fermenting mannite	ag	ag	ag	a	0	ac	1	
Not fermenting glucose	ag	0	ag	ag	0	ac	1	
Fermenting lactose but not milk	ag	ag	ag	ag	ag	a	1	
	ag	ag	ag	ag	0	a	1	
	ag	ag	ag	ag	0	0	1	
	ag	ag	ag	ag	ag	0	1	
Not fermenting lactose	ag	ag	0	ag	ag	a	2	
	ag	ag	0	ag	0	0	2	
	ag	ag	a	ag	a	a	1	
	ag	ag	0	a	a	a	1	
	ag	a	0	a	a	a	1	
	ag	a	a	a	a	ac	1	
	ag	ag	0	ag	0	ac	1	
	ag	ag	a	ag	0	ac	1	
Other aberrant organisms	a	a	a	a	a	ac	1	
	a	a	a	a	a	a	1	
	a	a	a	a	a	0	1	
	a	a	a	a	0	ac	1	
	a	a	a	a	0	a	1	
	a	a	a	0	0	ac	1	
	a	a	a	0	a	a	2	
	a	a	0	a	a	a	1	
	a	a	0	0	a	a	1	
	a	a	0	0	0	ac	2	
	a	a	0	0	0	0	2	
0	a	a	a	0	a	ac	1	
0	0	0	0	0	0	ac	1	

ag=acid and gas,

ac=acid and clot,

a=acid,

0=no reaction.

studied. In this connection it may be of interest to give here the reactions of 225 bacteria isolated from cultures of shellfish in neutral-red, bile salt, lactose agar. These are all of which I have kept the records. After plating out, several colonies were selected from each plate, and these were inoculated on the surface of nutrient agar contained in slant tubes. The pure subcultures were incubated for 24 hours and then the tertiary subcultures were made. The latter were incubated for 48 hours, but dulcrite cultures were usually kept for a week, and sometimes were kept at room temperature after incubation at 39° C. Cultures tested for the Voges and Proskauer reaction were kept at room temperature for a week.

Most of the reactions included in MacConkey's Table have been included in the methods of differentiation of these organisms. Apparently we may regard the fermentation of cane-sugar as non-essential in the identification of *Bacillus coli*. We find therefore that about 75% of the organisms cultivated produce acid and gas in (1) bile salt broth, (2) glucose broth, (3) lactose broth, (4) mannite broth, and (5) clot and acidify litmus milk. About 5% did not ferment lactose and were certainly not *B. coli*. A few gave the equivocal result of fermenting lactose but not milk, and *vice versa*. The majority, however, prove to be forms nearly allied to the typical colon bacillus.

In later analyses I employed the other fermentation tests recommended by MacConkey (1906) but with less satisfactory results. Table II gives the results of 72 series of reactions, most of which are those suggested in MacConkey's paper. The gas-ratio and the motility were not systematically observed.

Approximately the same percentage of the organisms subcultured conform to the principal characters given in MacConkey's first paper (1901). But if we regard the fermentation of dulcrite, and the non-fermentation of inulin and adonite, as essential characters of the colon bacillus, only about 18% of the organisms isolated and identified provisionally as such can be so diagnosed. This may be possibly the real proportion, but the results are in other respects less satisfactory, and productive of some confusion.

Among these 72 organisms there are no less than 17 distinct categories—assuming for the moment that every combination of all or some of the reactions possible is indicative of a distinct species of organism. With the exception of those in the two top lines, and those fermenting dulcrite, but not inulin nor adonite, there is considerable difficulty in classifying the forms according to the cultural reactions

displayed. Nevertheless the distribution of the reaction-frequencies appears to be determined by some manner of grouping of the organisms according to their biological characters, for there are two main categories—1, 2 and 9, 10—and the frequencies of these and the other groups do not conform to the normal law of error.

TABLE II.

*Reactions of 72 organisms isolated from Cultures on Neutral-Red, Bile Salt, Lactose Agar.*

	Bile salt broth	Glucose broth	Lactose broth	Mannite broth	Cane sugar broth	Dulcite broth	Inulin broth	Adonite broth	Voges & Proskauer Milk reaction	Total	%
1	ag	ag	ag	ag	0	0	0	0	ac	0	17
2	ag	ag	ag	ag	0	0	a	0	ac	0	11
3	ag	ag	ag	ag	0	0	0	a	ac	0	1
4	ag	ag	ag	ag	0	0	a	a	ac	0	1
5	ag	ag	ag	ag	ag	0	0	0	ac	0	1
6	ag	ag	ag	ag	ag	0	a	ag	ac	0	3
7	ag	ag	ag	ag	0	0	0	ag	ac	0	3
8	ag	ag	ag	ag	0	0	a	ag	ac	0	3
9	ag	ag	ag	ag	0	ag	a	0	ac	a	7
10	ag	ag	ag	ag	ag	ag	0	0	ac	0	6
11	ag	ag	ag	ag	ag	ag	a	ag	ac	0	1
12	ag	ag	ag	ag	0	ag	a	ag	ac	0	2
13	ag	ag	ag	ag	ag	ag	ag	0	ac	0	1
14	ag	ag	ag	ag	ag	ag	a	0	ac	0	1
15	ag	ag	ag	ag	ag	a	a	0	ac	0	1
16	ag	ag	ag	ag	a	a	a	0	ac	0	1
17	ag	ag	ag	a	a	a	a	0	ac	0	1
18	ag	ag	ag	ag	0	a	a	a	ac	0	1
19	ag	ag	ag	ag	0	a	a	ag	ac	0	1
20	ag	ag	a	ag	ag	ag	a	0	0	+	1
21	ag	ag	a	ag	a	0	0	ag	a	+	1
22	ag	ag	a	ag	ag	a	a	0	0	0	1
23	a	a	ag	ag	ag	0	a	a	ac	0	2
24	a	a	ag	ag	0	0	0	0	ac	0	1
25	a	a	a	a	a	0	0	ag	ac	0	1
26	a	a	a	a	a	0	a	a	0	0	1
27	a	a	a	a	ag	0	a	0	ac	0	1

Obviously such results as these place us on the horns of a dilemma. It is absolutely necessary, in justice to the interests involved, that the approval or condemnation of a shellfish laying, or a large consignment of fish, should be based on the results of examination of a fairly large number of individual specimens—and probably also of a number of

separate samples, taken *in situ*: that is, if the bacteriological results are held to be sufficient evidence of the grade of pollution. A certain number of bacteria resembling the colon bacillus are estimated as being present in each shellfish, and a fraction of these must be subcultured in order that this provisional identification may be confirmed. In the analyses made by myself ten mussels, or other shellfish, usually formed a sample. Each mollusc was examined individually so as to get an idea of the range of variability, and often a strictly quantitative estimation of the numbers of bacteria of the intestinal group present in the whole body of a shellfish was made by method two (see p. 422). Then a certain number—usually ten—of the colonies provisionally identified as *B. coli* were subcultured and examined in detail. Often it was quite essential to take several samples so as to study the influence of season, winds, tides, or other conditions. If, say, five of the ten sample colonies answered to the tests for *B. coli* it was assumed that about half of all those isolated in the primary cultures, and identified as "intestinal bacteria," were really colon bacilli. The labour of such an investigation is considerable and one is almost compelled to adopt the minimum number of tests necessary for the diagnosis of *B. coli*. On the other hand it appears that more tests are necessary than was formerly supposed for this end; and if one hesitates to make use of them he runs the risk of identifying as the colon bacillus organisms which do not possess the significance of this form; and in applying the conclusions deducible from this diagnosis with consequences detrimental to the shellfish industry.

#### Variability of reaction.

It might be expected that the multiplication of the reactions employed in the identification of organisms of the intestinal group would lead to a greater differentiation of species. But the trouble is that the number of apparently distinct forms becomes large—so large as to appear to make it *a priori* improbable that they can all be separate species. The difficulty is analogous to that which has occasionally arisen in purely zoological investigation as the result of the work of systematists endowed with more than the average analytical powers. The "splitters" have burdened the literature with a host of names which have come to possess only historical interest; and have called forth the "lumpers" whose tendency has been to confuse together well-defined species. In later days science has been rescued

from both by the mathematical study of variation. Now it appears to an outsider who has to study bacteriological literature that there has been a tendency towards the creation of ill-defined species of bacteria by the pathologists, and that a number of those described have really no separate identity, in the meaning of the term "specific identity" as employed by the systematic zoologists and botanists. On the other hand there appears to be a tendency towards the confusion of probably separate organisms on the part of bacteriologists who have to employ easy routine methods of identification of organisms of economic significance.

If one were to isolate faecal *Bacillus coli*, taking great care to secure a number of colonies resulting from the division of one original organism, and then proceed to cultivate these separately, using identical series of tests for each, would one obtain precisely the same series of results for each of the colonies studied? I think it is very doubtful. In that case one would prove the existence of metabolic variability in the species studied—a result which is indeed more than probable. If a great number of organisms isolated from different strains of *Bacillus coli* were so studied<sup>1</sup> we should be able to form "frequency curves" expressing the probability of any particular series of reactions being associated with an organism of the type of *Bacillus coli*; and we should be able to say what particular deviation from this general series of reactions should be regarded as removing the organism from this category of bacteria. It appears not improbable that such is the only method by which we should be able to devise a series of tests which might be applied with reasonable probability to the identification of the bacteria obtained from polluted sea-water or shellfish.

It seems to be clearly proved that organisms of the typhoid-colic type do not normally inhabit sea-water, or the tissues of marine shellfish—a conclusion which emerges from the experimental work of Klein (1905), Herdman and Boyce (1899), and others. Placed in clean sea-water both *B. typhosus* and *coli* cease to reproduce and soon disappear. If this is the case with mammalian intestinal organisms in general when they enter the sea it is probable that "loss of attribute"—that is, changes in metabolic activity leading to the failure to produce one or other fermentation reactions—should precede this ultimate dissolution of the bacteria. As the result of such changes a certain proportion of the organisms sampled—a proportion variable with the precise conditions—will fail to respond to one or more of the tests applied. I have

<sup>1</sup> This has already been done, to some extent, by Dr Houston (1904, 1906).

noticed—though I have no extensive series of results to quote—that there is sometimes a general similarity in reaction between the bacteria isolated from one particular sample, when compared with the results of other samples taken under different conditions. One may explain this on the hypothesis that the differences in reaction were due to a longer or shorter sojourn in the sea; and consequent loss of fermentation powers. It is universally recognised that recent pollution is far more significant than pollution of remote date. It is very difficult to isolate *Bacillus typhosus* from shellfish, though the employment of such media as that of Drigalski and Conrad (1902), or the neutral-red, bile salt, lactose agar used in the present investigations, renders the separation of the organisms in question by no means difficult<sup>1</sup>.

Would a known strain of faecal *Bacillus coli* multiply in a sterile medium resembling as much as possible the juices of the alimentary canal of the mussel or oyster? Probably it might do so for a short time, but it is not certain that the organisms thus produced would give all the reactions exhibited by the original strain of bacillus. The ideal way to carry out the experiment would be to infect shellfish known to be perfectly clean and sterile (so far as sewage organisms are concerned). But it appears that bacteria of intestinal origin rapidly disappear when

<sup>1</sup> I have only succeeded once in isolating what appears to have been *Bacillus typhosus* from shellfish (1907). The laying from which the mussels were obtained was situated immediately round a sewer outfall which continuously discharged crude sewage. The sewer served a seaside resort having a large fluctuating holiday population. The time was June. The reactions of the organism were as follows:

It formed a round, slightly raised, translucent, colourless colony of about two mm. in diameter, on neutral-red, bile salt, lactose agar after twenty-four hours' incubation at 41° C.

It was very motile.

It formed acid and gas in bile salt glucose broth;

acid only in glucose litmus broth;

a slight discolouration in lactose litmus broth;

acid only in mannite broth;

acid only in milk.

And it gave no reaction with cane sugar litmus broth.

It agglutinated—in a dilution of one in thirty—in a serum which gave a positive reaction with a known strain of *Bacillus typhosus*. (This test was made for me by Mr Lewis, of the Pathological Department in the University of Liverpool.)

The local Public Health Officers denied the existence of enteric fever in the locality, a condition which proves nothing, since convalescents or "carriers" may have been resident there and have been unknown.

It is however very probable that *B. typhosus* may have been present in several samples of shellfish examined by me, but the labour of isolating and examining all the colonies likely to have been produced by this organism is too great to admit of its being carried out as a matter of routine practice.

inoculated in shellfish or sea-water. If loss of attribute could be shown to ensue as a consequence of such procedure it is clear that the principle of "revivifying" microbes isolated from shellfish, by repeated subculture in media resembling as closely as possible those in which they have their natural habitat, should be applied. This has been suggested, but the counsel appears to be one of perfection, and probably not applicable as a matter of routine practice.

#### **Standards of permissible impurity.**

Responsible bacteriologists who have discussed the institution of such standards have generally hesitated to suggest them, except in a tentative manner. Nevertheless most of those who have had much experience in the analysis of shellfish must—unconsciously perhaps—have set up some sort of standard in relation to their own work; for it is only with reference to some criterion of impurity, or by the statement of some remedial measures, that the results of such investigations can be expressed in a form suitable for administrative purposes.

Topographical and epidemiological evidence are the only guides in the application of bacteriological results towards the erection of a standard of a permissible impurity. If a laying is evidently grossly polluted, and if faecal matter and sewage debris are found among the shellfish, no analysis is necessary; but a knowledge of the bacterial contents of the shellfish gives information which can be applied to the interpretation of bacteriological results in cases where the topographical conditions are unknown; as when, for instance, the shellfish have been taken from a shop, and the place of origin cannot be traced; and generally in cases where the report of the analysis is the only evidence obtainable. Epidemiological facts furnish a standard, for if a certain number of cases of illness can be traced year by year to a definite locality, and if the approximate numbers of *Bacillus coli* present in the shellfish taken from this locality are known, it is evident that such knowledge may be applied (with all due caution of course) to other localities where the distribution of disease by the shellfish has not been studied. It would give at all events *a priori* reasons for suspicion.

Bacteriological results if they are to be applied to the approval or rejection of shellfish must obviously be quantitative ones, for shellfish like the mussel nearly always contain bacteria which are of intestinal origin. My own experience has been that no sample of ten or more mussels can be examined without finding *Bacillus coli*, or at least some

organisms resembling this form. This statement applies, of course, to the layings of the west coast of England. Mussels are almost always found in creeks, or estuaries, where fresh water flows down from the land, and fishermen say that it is the "fresh" which is favourable to the growth of the molluscs. Certainly remarkable results have been obtained, in the way of the cultivation of these shellfish, by relaying them in such situations where they may obtain a plentiful supply of water of moderate salinity. It is, however, the substances contained in the water draining down from the land that are the factors producing the more rapid growth of the shellfish. The contained soluble carbon and nitrogen compounds may, in themselves, provide a source of food that can be utilised directly by the mussels; or these food stuffs may provide the pabulum for the diatoms or other protista—after resolution into inorganic compounds by fermentation and nitrifying bacteria—and the protista may then serve as a source of food for the molluscs. However this may be it seems to be generally the case that the largest mussels are those which have been grown where there is a certain proportion of sewage matters in the sea-water flowing over them<sup>1</sup>. The sea-water which is in contact with a mussel laying must therefore contain sewage bacteria, even although the laying may not be in immediate proximity to a sewer outfall. It may not always be possible to demonstrate the existence of such bacteria in one c.c. of the water but they will generally be found in larger volumes.

There are generally more of such organisms in the body of a mussel, or other shellfish, than are to be found in the same volume of the surrounding sea-water, as may be proved by making comparative cultures from the stomach contents, and from the water in the pallial cavity. Yet it appears that *Bacillus coli*, or its congeners, do not multiply in sea-water. Probably there is an initial multiplication in the tissues of the shellfish, after which the intestinal organisms begin to undergo loss of attribute, and their growth becomes inhibited. If the shellfish taken from a polluted place were put into perfectly clean sea-water and kept for a sufficient time it is probable that intestinal organisms would disappear entirely from their tissues. But in natural conditions there must be a continual reinfection of the molluscs.

Shellfish taken from layings which may be supposed to be outside the influence of sewer outfalls may contain appreciable numbers of sewage bacteria. I may refer to the case of a mussel bed at Roosebeck, in Morecambe Bay, which I examined and reported upon in 1906 (1907).

<sup>1</sup> A statement which has not, however, universal application.

This laying is situated immediately to the north of the mouth of Barrow Channel, and the direction of the tidal streams, together with the existence of training walls, renders it very improbable that any of the polluted water passing down the Channel from Barrow-in-Furness can come near to the mussels. It is situated about eight miles from Ulverston which appears to be the only community from which sewage might possibly come into contact with the laying. But it is very probable that before the effluent from the outfalls at Ulverston could reach Roosebeck the sewage would be so largely diluted as to render the contamination of little significance. There are one or two small outfalls on the shore about a mile from the laying, but these may be neglected. Considering the topographical evidence one would say that the shellfish were, in all probability, quite clean. Yet I found that the numbers of intestinal bacteria isolated from about 0·2 c.c. of the stomach juices were in the cases of ten mussels 40, 65, 9, 9, 2, 64, 28, 13, 3, and 9—average 24·2.

#### Cleansing of polluted shellfish.

If polluted mussels be supplied with clean sea-water there is a rapid partial disappearance of the intestinal bacteria contained in their tissues. This is a direct inference from the work of Klein (1905) and others. It appears that oysters cleanse themselves, in such circumstances, more rapidly than mussels, and mussels more rapidly than cockles. In the summer of the present year I made some experiments designed to ascertain the period in which this partial cleansing of polluted mussels might be expected to take place. The shellfish were taken from an undoubtedly polluted area—one with regard to which there was direct epidemiological evidence of the transmission of enteric fever by means of the mussels taken therefrom. The topographical conditions were quite in accord with the meaning of the epidemiological results. These polluted mussels contained on the average 1900 intestinal bacteria per shellfish<sup>1</sup>. They were put into large wooden boxes which were then deposited on the beach in a situation where they were half a mile from the nearest sewer outfall: further, they were placed about half-way up the beach so that they were uncovered when the tide had ebbed to the extent of about one half of its usual period. The water which they received, while not unpolluted, was reasonably clean. The

<sup>1</sup> The plates also contained about 25 colourless colonies each. None of these colonies appeared on the plates made from the relaid shellfish.

experiment was not made with the object of bringing about a complete disappearance of intestinal bacteria, but was intended to suggest some practical means, with reference to the particular locality, of storing the shellfish taken from the polluted beds in the vicinity, for such a time as would enable them to eliminate the greater proportion of the contained sewage bacteria. After four complete days a sample of the mussels was taken and it was found that the number of contained bacteria had been reduced to about 150 per shellfish—a reduction of about 93 per cent. They were left for about three times this period, but it was found that the further reduction of the contained bacteria was slight. For all practical purposes the cleansing had taken place during the first four days during which the shellfish had been relaid. In the course of the experiment a short gale sprung up and one of the boxes containing the mussels went adrift, with the result that it sailed into highly polluted water and the shellfish became reinfected to about their original degree. The box was replaced in the same place and it was again found that the bacteria were eliminated in four days to the same extent as before.

These two series of results indicate the possibility of setting up a standard of bacteriological impurity which may be regarded as of little importance from the point of view of the public health. It is extremely unlikely that the Roosebeck mussels are contaminated to such a degree as need cause any apprehension of disease as the result of their use as human food. No cases of disease have ever been traced to the use of these shellfish. In the case of the cleansing experiments a residue of intestinal bacteria remained after about twelve days' sojourn in reasonably clean sea-water, which amounted to about 150 per mussel. Now Klein (1905, pp. 50—53) showed that mussels containing the enormous number of six millions of *Bacillus typhosus* per shellfish were cleaned to the extent that one mollusc contained about 14,000 bacilli after seven days, simply by a daily change of the sea-water in which the shellfish were contained. The numbers of this bacillus that could possibly be taken up by a mussel or oyster in natural conditions could not be expected ever to reach the number of millions; and if even the rate of cleansing experienced in Klein's experiments were to hold good in the sea, under the conditions of the experiments referred to above, it may be expected that any pathogenic bacteria imbibed by the molluscs would be eliminated. We have seen that even after the short period of four days the reduction was considerable.

But it appears that the institution of a standard depends on the consideration of both topographical and epidemiological evidence; and that the results of bacteriological analyses are to be interpreted in the light of such information. This makes it doubtful whether we are ever quite justified in applying the results of analyses alone in administrative routine. It would be very desirable if a local authority were able to reject or approve a consignment of shellfish on the evidence of a report by a bacteriologist, for a good deal of trouble would thus be avoided. Unfortunately the adoption of such procedure would in many cases result in hardship to the fishermen, while the real source of pollution might not always be traced.

#### **Summary and Conclusions.**

(1) At present no public authority possesses legal power to deal with the question of the contamination of shellfish.

(2) It is not sufficient to test shellfish exposed for sale in a market or shop. These may have been contaminated subsequent to removal from the fishery; and multiplication of the contained bacteria may have taken place. The results of such analyses may lead to unjustifiable condemnation of a laying. It is essential that a topographical examination should be made and that samples for analysis should be taken from the laying itself.

(3) In the case of natural shellfish beds there is so much variability in the conditions with regard to the susceptibility to pollution that a fairly large number of the animals must be examined. The labour of the analyses is therefore so great that the development of some simple routine test for faecal contamination is most desirable. Since most natural shellfish layings are situated within the "sewage zone," and therefore contain *B. coli*, quantitative results are essential.

(4) There are considerable differences in practical routine work in regard to the methods of isolation of intestinal organisms from shellfish; and also with respect to the number and nature of the reactions necessary for the identification of *B. coli*. It is desirable that some generally recognised series of tests should be uniformly adopted by bacteriologists engaged in such work. Further, different micro-organisms, possibly of varying degrees of significance as indicators of faecal contamination, may have been confused. There is possibly some variation in cultural characters in *B. coli*, and investigation of

this variability is desirable. Investigation of the changes in cultural reactions undergone by intestinal organisms when entering the sea, or the tissues of marine shellfish, is also very desirable.

(5) Remedial measures other than the simple closure of a contaminated laying might be suggested. It is possible to subject the shellfish to treatment which will cause them to clean themselves of contained sewage bacteria. The source of the pollution may be removed; and sterilisation of the shellfish may be practised.

## EXPLANATION OF PLATE II.

Fig. 1. Culture from 1 c.c. of an emulsion of the bodies of 5 mussels made up to 250 c.c. ( $=0\cdot02$  mussel). Neutral-red, bile salt, lactose agar was used for isolation. Incubated for 20 hours at  $42^\circ$  C. and kept for three days at room temperature before being photographed. These mussels were badly polluted.

Fig. 2. A similar culture ( $0\cdot02$  mussel) made precisely as above from five of the same lot of mussels after they had been kept in unpolluted sea-water, in the open, for four days. The plates represent fairly the difference in bacterial contents that may be expected from such treatment.

(The photographs are by my colleague, Mr A. Scott.)

## REFERENCES.

- DRIGALSKI, V. and CONRADI, H. (1902). Ueber ein Verfahren zum Nachweis der Typhusbacillen. *Zeitschr. f. Hygiene*, Vol. XXXIX. p. 283, 1902.
- GRÜNBAUM, A. S. and HUME, E. H. (1902). Note on Media for distinguishing *B. coli*, *B. typhosus* and related species. *British Medical Journal*, Vol. II. pp. 1473, 1474, June, 1902.
- HERDMAN, W. A. and BOYCE, R. (1899). Oysters and Disease. *Lancashire Sea-Fisheries Memoir I*. London, 1899. (Reprinted in *Thompson-Yates Laboratories Reports*, Vol. II. Reprints and Reports, 1898-9, 1900.)
- HOUSTON, A. C. (1904, a). *Fourth Report of the Royal Commission on Sewage Disposal*, Vol. III. [Cd. 1885], 1904.
- (1904, b). Report on the bacteriological examination of the normal stools of healthy persons. *Supplement to the Report of the Medical Officer to the Local Government Board for 1902-3*, Appendix, pp. 511-566, 1904.
- (1904, c). The bacteriological examination of Oysters and Estuarial Waters. *Journal of Hygiene*, Vol. IV. pp. 173-200, 1904.
- (1905). Report on the intestinal contents in sea-fowls and fish. *Supplement to the Rept. of the Medical Officer to the Local Government Board for 1903-4*, pp. 472-608, 1905.

- JOHNSTONE, J. (1905). Bacteriological investigations in relation to shellfish pollution by sewage matter. *Rept. Lancashire Sea-Fisheries Laboratory for 1904.* (In *Trans. Liverpool Biol. Soc.* Vol. xix. pp. 242-270, 1905.)
- (1907). Report on various bacteriological analyses of mussels from Lancashire and Wales. *Ibid.* Vol. xxi. pp. 328-370, 1907.
- (1909). Bacteriological investigations in relation to shellfish pollution. *Ibid.* Vol. xxiii. pp. 203-227, 1909.
- KLEIN, E. (1905). Experiments and observations on the vitality of the bacillus of Typhoid Fever and of sewage microbes in oysters and other shellfish. Published by the Fishmongers' Company, London, 1905, p. 79.
- MACCONKEY, A. T. and HILL, C. A. (1901). Bile Salt Broth. A simple test for faecal contamination. *Thompson-Yates Laboratories Repts.* Vol. iv. Pt. I. pp. 151-165, 1901.
- (1906). A contribution to the Bacteriology of Milk. *Journal of Hygiene,* Vol. vi. pp. 385-407, July, 1906.
- (1908). Bile Salt Media and their advantages in some bacteriological examinations. *Journal of Hygiene,* Vol. viii. pp. 322-334, 1908.
- MCWEENEY, E. J. (1904). Report on the bacterioscopic examination of samples taken from shellfish layings. In *Report on the shellfish layings on the Irish coast as regards their liability to sewage contamination.* [Cd. 1900], 1904, pp. 85-148.

## PUBLICATIONS RECEIVED.

### BOOKS.

DIEUDONNÉ, A. (1909). *Bacterial Food Poisoning*. A concise exposition of the etiology, bacteriology, symptomatology, prophylaxis and treatment of so-called ptomaine poisoning. (Translated and Edited, with Additions, by Dr C. F. Bolduan.) New York: E. B. Treat & Co., 128 pp., 21×14 cm. Price \$1.00. Cloth.

Dieudonné's valuable little treatise appeared in 1908 and the translation reached us in July 1909. Dr Bolduan has made certain additions relating to prophylaxis applicable to American conditions and has gone further into details of treatment. The material has been somewhat rearranged and an index added. The nine chapters into which the book is divided concern themselves respectively with, (1) meat poisoning due to diseased meat, (2) to decayed meat, (3) sausages, (4) poisoning due to fish and molluscs, (5) to cheese, (6) to ice cream and puddings, (7) to potatoes, (8) canned foods, (9) metals. In each chapter the etiology, symptoms, diagnosis, including bacteriology, prophylaxis and treatment are given. About 100 references are given in the bibliography. The book will prove very useful to those not familiar with the scattered literature on the subject.

ELLIS, D. (1909). *Outlines of Bacteriology* (Technical and Agricultural). London: Longmans, Green & Co., 262 pp., 134 illustrations, 22×14 cm. Price 7/6 net. Cloth.

This book is intended for students of technical and agricultural bacteriology and in consequence the pathogenic bacteria are treated somewhat cursorily. In a book of such an elementary character it is difficult to introduce more than the essentials, and the author appears to have done this with judgment.

FRIEDEMANN, U. (1910). *Taschenbuch der Immunitätslehre*. Leipzig: Joh. Ambrosius Barth, 140 pp., 17·5×11·5 cm. Price 4 Marks.

This little book contains much useful information condensed into a few pages and without pretending to be complete it gives the main methods used in experimental and clinical work on immunity: Technique in relation to animals; tests with various antibodies, haemolysins, agglutinins, bactericidal substances, precipitins, etc. The book can be safely recommended to those desiring information on our present methods.

JAMES, S. P. (1909). *Smallpox and Vaccination in British India*. 105 pp., 14 diagrams, 25×19 cm. Price 7/6 net, in India 6 Rupees. London and Calcutta: Thacker, Spink & Co., 2 Creed Lane, London.

The author presents us with a very interesting history of smallpox inoculation and of the introduction of vaccination into India and details some of the administrative difficulties that have had to be encountered. By means of a series of graphic charts the author demonstrates the beneficial effects that have

followed vaccination, especially in certain parts of India and in the Army both European and native. The author has acquitted himself well of an exceedingly difficult task and the publication constitutes a valuable contribution to the literature of smallpox.

- NEWELL, A. G. (1909). *Blackwater Fever. (Bilious Malignant Tertian Ague.)* London: John Bale, Sons & Danielsson, Ltd., 126 pp., 1 plate (frontispiece), 22 × 14 cm. Price 5/- net. Cloth.

The author gives an account of his experiences with blackwater fever during nine years spent in highly malarial parts of Bengal. He discusses the views held regarding the etiology of blackwater and describes the symptoms, treatment, etc. He gives full clinical histories of five cases including his own. A series of appendices deal with cognate matters.

- OSLOWSKI, DR (1909). *Die Schönheitspflege. Für Ärzte und gebildete Laien.* 2nd ed., 120 pp., 25 text-figs. Würzburg: Curt Kabitsch (A. Stuber's Verlag). Price 2.50 Marks. Linen.

This booklet, as the sub-title indicates, is written for medical men and educated laymen. It concerns itself with the care of the body and the preservation of its natural beauty. It is an attempt to deal with the subject in a scientific manner and is directed against charlatanism. The book contains much interesting information in condensed form. From the fact that it has already reached a second edition it is obvious that it has met with a demand.

- POWELL, A. E. (28. X. 1909). *Food and Health.* Methuen & Co., London, W.C. 266 pp., 19 × 13 cm. Price 3/6 net. Cloth.

The author, a Lieutenant in the Royal Engineers, has compiled this book for ordinary laymen. The book contains a good deal of varied information especially regarding diet, ancient and modern, in many parts of the world. The author inclines to vegetarianism.

- RAYNES, F. W. (1909). *Domestic Sanitary Engineering and Plumbing.* London: Longmans, Green & Co., 474 pp., 277 illustrations, 22 × 14 cm. Price 10/6 net. Cloth.

This book, as stated in the subtitle, deals with domestic water supplies, pump and hydraulic ram work, hydraulics, sanitary work, heating by low pressure, hot water, and external plumbing work. Although intended for students preparing for examination the book should prove of value to a wider circle of readers. The book contains a good many excellent text-figures and useful formulae and altogether presents an excellent appearance.

- WANHILL, C. F. and BEVERIDGE, W. W. O. (1909). *The Sanitary Officer's Handbook of Practical Hygiene.* London: E. Arnold, 150 pp., 19 × 13 cm. Price 5/- net. Cloth.

Majors Wanhill and Beveridge of the Royal Army Medical Corps, the authors of this little book, hold respectively the positions of Assistant Professor of Hygiene in the Royal Army Medical College and of Analyst to the Army Medical Advisory Board. The plan of the book is that on which the "training in the Hygiene Laboratories, Royal Army Medical College, is carried out and there found to be satisfactory both for military purposes and for preparation for the examinations for the Diploma in Public Health." This being the case, the book will no doubt meet with a considerable demand.

WARD, A. R. (1909). *Pure Milk and the Public Health.* A manual of Milk and Dairy Inspection. Ithaca, N.Y.: Taylor and Carpenter, 218 pp., 17 illustrations, 23 x 16 cm. Price \$2 net. Cloth.

In the volume before us, which is dedicated to Veranus A. Moore, the author has brought together facts which will necessarily interest the health officer and "others directly concerned in the crusade for better milk." It is assumed by the author that the reader possesses a general knowledge of bacteriology. The literature of the subject is fully cited in the text and full references are given to the papers quoted. The book is divided into eleven chapters: (1) The contamination of Milk, (2) Changes in Milk caused by Bacteria, (3) Epidemic Diseases transmitted by Milk, (4) Bovine Tuberculosis and other Cattle Diseases, (5) Municipal Sanitary Control of Milk, (6) Pasteurization of Milk, (7) Microscopic Tests of Milk, (8) Bacteriological Examination of Milk, (9) Certified Milk, (10) Analysis of Milk, (11) Adulteration of Milk. An Appendix includes Dairy Ordinances of Berkeley, California, etc. The author is Assistant Professor of Bacteriology and Director of the State Hygienic Laboratory, University of California, which enables him to write with authority. Two chapters are from the pen of M. E. Jaffa, Professor of Nutrition and Director of the State Food and Drug Laboratory, University of California. The writer has condensed a great deal of information into the work, which is a valuable addition to the literature on milk.

*The Influence of Heredity on Disease, with special reference to Tuberculosis, Cancer and Diseases of the Nervous System.* A discussion opened by Sir W. S. Church, Bt., K.C.B., M.D., Sir W. R. Gowers, M.D., F.R.S., A. Latham, M.D., and E. F. Bashford, M.D. 142 pp.+xii. Longmans, Green & Co., London. 26 x 19 cm. Price 4/6 net. Cloth. (Reprinted from *Proc. Roy. Soc. of Medicine*, London, Vol. II.)

As stated in the preface to the volume "in view of the importance of determining the influence of heredity as an etiological factor, the Council of the Royal Society of Medicine have thought it right to publish in a separate volume the discussion on this subject held at the Society's rooms" in November-December 1908. There are many who will welcome the book for the reason above stated. An excellent index prepared by Mr A. L. Clarke materially adds to the value of the book.

### BROCHURES.

BASU, B. D. (1909). *The Dietetic Treatment of Diabetes.* Allahabad : The Panini Office, Bhuvaneshvari Ashram. 40 pp., 19 x 13 cm. Price Rs. 1-8-0. Cloth.

BURRI, R. (1909). *Das Tuscheverfahren als einfaches Mittel zur Lösung einiger schwierigen Aufgaben der Bakterioskopie* (Absolute Reinkultur, Spirochaeten-nachweis u.a.m.), 41 pp., 3 plates, 3 text-figs. Jena: Verlag von Gustav Fischer. Price 3 Marks. 26 x 17 cm.

The author describes what promises to be a very useful and rapid method of examining fluids, secretions, etc. containing bacteria, spirochaetes (*Syphilis*) or blood containing protozoa. The method consists in mixing the material to be examined with Indian ink and spreading it upon a slide. *The unstained*

*spirochaetes etc. stand out clearly on the black background* and when photographed give excellent lantern slides, the negative appearing as a diapositive. The method applies particularly well to cultures starting from single bacteria. The author is to be congratulated on the discovery of a valuable method.

HART, A. H. (1909). *How to Cut the Drug Bill.* London: John Bale, Sons and Danielsson, Ltd., 83-91 Great Titchfield Street, Oxford Street, W. 47 pp. Price 2/6.

Intended for practitioners who do their own dispensing. Lessens annual cost of drugs dispensed by 20-50% by cutting out most alcoholic preparations, etc.

IAROTZKY, A. (1908). *Der Idealismus als lebenerhaltendes Prinzip.* Wiesbaden: Verlag von J. F. Bergmann, 147 pp.

PAGET, STEPHEN (1909). *The Case against Christian Science.* Cassell and Company, Ltd., London, 36 pp. Paper, price 6d. net. 20 x 13 cm.

Contains papers read at the Church Congress, Swansea, and at the Congregational Union's Meeting at Sheffield. Very entertaining reading.

*The Medical Inspection of School Children.* A Series of Lectures delivered at the West London Post-Graduate College. 62 pp., price 1/- net. London: "The Medical Officer."

This pamphlet includes: The general scheme of Inspection by A. SAUNDERS, M.B.—Examination of skin disease by P. S. ABRAHAM, M.D.—Examination of the Eyes by K. SCOTT, M.D.—Examination of the Ear, Nose, and Throat by H. J. DAVIS, M.B.—Examination of the Teeth by H. LL. WILLIAMS.

## REPORTS.

*Australasian Medical Congress. Transactions of the 8th session held at Melbourne, Victoria, October 1908.* Edited by Alex. Lewers, M.R.C.S. etc. Victoria: J. Kemp, Gov't. Printer, Melbourne, 1909.

Vol. I. Transactions: Conjoint Sectional Meetings—Section of Medicine—Section of Surgery. 421 pp.

Vol. II. Sections of Obstetrics and Gynaecology—Public Health—Anatomy and Physiology—Pathology and Bacteriology. 393 pp.

Vol. III. Sections for Diseases of Children—Diseases of the Eye, Ear and Throat—Skin and Radiotherapy—Neurology—Military and Naval Hygiene. Sectional Index. 430 pp.

The following papers appear in the Section of Public Health: President's address by J. MASON—Syphilis from the standpoint of Preventive Medicine (1) by A. J. TURNER and (2) by B. B. HAM—Colonization of Tropical Australia by R. ARTHUR—Periodicity in Epidemic Disease by J. JAMIESON—Medical examination of School Children in Tasmania by S. HALLEY—Examination of School Children by A. H. CLARKE—The Maori by M. POMARE—The Medical Officer of Health by A. THOMPSON—Development of Public Health work in Tasmania by J. S. C. ELKINGTON—Sanitary Administration and Reform by E. G. LEGER-ERSON—Public Health Conscience by M. BOOTH—Cooperation between Health Authorities by J. S. C. ELKINGTON—Infant Mortality in a Mining Centre by B. S. COWEN—Petroleum in Insect-borne Diseases by J. S. PURDY—City of Pahran Refuse Destructor by R. H. FETHER-



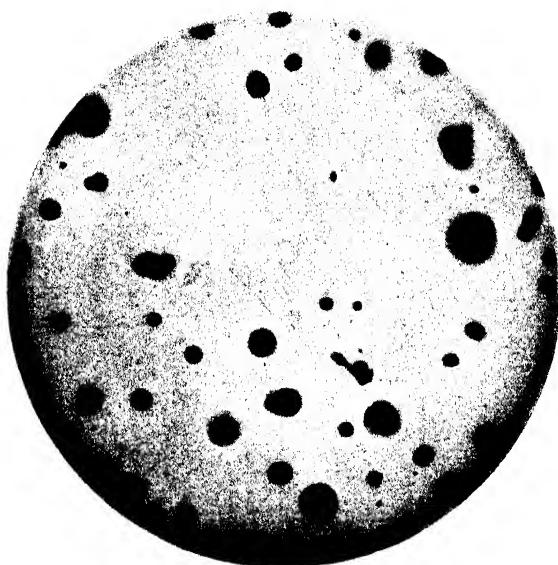


Fig. 1.

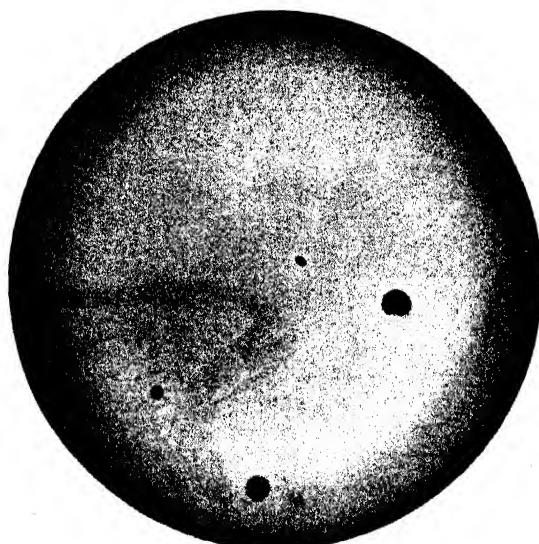


Fig. 2.

- STON and W. CALDER—Statistics of Tuberculosis by G. H. KNIBBS—Statistical Opportunities of the Medical Profession by G. H. KNIBBS—Tuberculosis frequency curves by G. H. KNIBBS.
- CARPENTER, E. G. (1909). *Rapport sur la Mortalité Infantile*. (Municipalité d'Alexandrie.) Alexandria: Société de Publications Égyptiennes, 40 pp.
- CHAPIN, C. V. (1909). *Fifty-fourth Annual Report upon the Births, Marriages, and Deaths in the City of Providence for the year 1908*. 133 pp. City Document No. 20. The Providence Press: Snow and Farnham Co., City Printers.
- CHAPIN, C. V. (1909). *Twenty-sixth Annual Report of the Superintendent of Health of the City of Providence for the year 1908*. 115 pp. City Document No. 14. The Providence Press: Snow and Farnham Co., City Printers.
- DAVIES, D. S. (1909). *Annual Report of the Medical Officer of Health, and of the General Medical Superintendent of the City Hospitals for 1908*. (City and County of Bristol.) Further report on "carrier" Typhoid. Bristol: Bennett Brothers. 178 pp. Boards.
- DAVIES, D. S. and HEAVEN, J. C. (1909). *Annual Report of the Medical Officers of Health and of the Chief Port Sanitary Inspector, for the year 1908, including Report on Canal Boat Inspection*. Bristol: Bennett Brothers Ltd. 49 pp.
- ELKINGTON, J. S. C. (1909). *Department of Public Health, Tasmania. Annual Report for the year 1908-9*. Hobart, Tasmania: John Vail, Government Printer. 13 pp.
- FREMANTLE, F. E. (26. II. 1909). *First Annual Report of the School Medical Officer on the Medical Inspection of Elementary School Children in respect of the year ended 31st December, 1908*. 28 pp. Hertfordshire County Council.
- HILL, E. (1909). *Report of the Health Officer of the Colony of Natal for the year ended 31st September, 1908*. Pietermaritzburg: Times Printing and Publishing Co. Ltd., 45 pp., price 9d. 33 × 21 cm.
- HOUSTON, A. C. (II. 1909). The storage of Raw River Water antecedent to Filtration. *Third Report on research work*. Metropolitan Water Board. Metropolitan Water Board, London, 47 pp. 33 × 21 cm.
- HOUSTON, A. C. (VI. 1909). Report on the results of the Chemical and Bacteriological Examination of the London Waters for the Twelve Months ended 31st March, 1909. *Metropolitan Water Board: Third Annual Report*. 42 pp.
- HOUSTON, A. C. (VI. 1909). The vitality of the Cholera Vibrio in artificially infected samples of raw Thames, Lee and New River Water, with special reference to the question of storage. *Metropolitan Water Board: Fourth Research Report*. 18 pp.
- II. *Internationale wissenschaftliche Lepra-Konferenz*, abgehalten v. 16 bis 19 August 1909, in Bergen (Norwegen). Mitteilungen u. Verhandl. herausg. v. Dr. H. P. Lie (General-Sekretär) Bd. 1, 153 pp., 2 portraits and 3 maps. Leipzig: Joh. Ambr. Barth; Paris: Masson & Cie; London: Williams & Norgate; New York: Lamke & Buechner. Price (Cloth) 8 Marks. 28 × 20 cm.  
 The first volume of the reports and papers of the second international scientific conference on Leprosy is adorned by two excellent portraits of Danielssen and Boeck. Ehlers and Verdier have written a chapter on the geographical distribution of Leprosy, G. Armauer Hansen and H. P. Lie give account of the history of Leprosy in Norway (2 maps and several figures), whilst Sederholm, Bjarnhjedinsen and Fagerland write respectively on

Leprosy in Sweden (1 map), Iceland and Finland. It seems scarcely necessary to say that the publication is one of the first importance to leprologists all over the world. The printing and illustrations leave nothing to be desired.

- KOCH, R., BECK, M. and KLEINE, F. (1909). *Bericht über die Tätigkeit der zur Erforschung der Schlafkrankheit im Jahre 1906-07 nach Ostafrika entsandten Kommission.* 319 pp., 5 pls., 180 figs. (Also published in *Arb. a. d. Kaiserl. Gesundheitsamte*, xxxi., as the 1st Fasciculus.) Berlin: Verlag von Julius Springer.

The report on sleeping sickness by the German Commission is one of the first importance and should be consulted by all who concern themselves with the disease. It deals with the etiology, symptomatology, treatment and prevention of the disease and includes, besides maps, a large number of most excellent illustrations. The clinical histories of 301 cases of sleeping sickness are included in the appendix. The report is a monument of industry and incorporates a very large amount of valuable original work.

- LISTON, W. G. (1909). *Report of the Bombay Bacteriological Laboratory for the year 1908.* Bombay: Printed at the Government Central Press. 15 pp. Price 5 annas, or 6d.

- E. Merck's annual report of recent advances in Pharmaceutical Chemistry and Therapeutics, 1908, vol. xxii., 348 pp. (Published VIII. 1909.) E. Merck, Chemical Works, Darmstadt. Price 1/6.

Copies of this publication, of which there are a limited number, may be obtained free by medical men on application to E. Merck, 16 Jewry St, London. The report brings together a great deal of useful information in the form of abstracts of the literature which appeared in 1908.

- Rapports à M. le Préfet, Préfecture du Dép. de la Seine.* Direction des Affaires Municipales. Bureau Administratif des Services d'Hygiène de la Ville de Paris. Paris: Imprimerie et Librairie Centrales des Chemins de Fer. Imprimerie Chaix, Rue Bergère 20.

(a) (1908, 70 pp.) 1<sup>o</sup> Sur quelques îlots de maisons tuberculeuses (20 déc. 1904);

2<sup>o</sup> Sur la répartition de la mortalité par la tuberculose pulmonaire dans les maisons de Paris, du 1 jan. 1894 au 31 déc. 1904 (20 mars 1905);

3<sup>o</sup> Sur la répartition de la tuberculose pulmonaire dans les maisons de Paris, pendant l'année 1905 (19 mars 1906);

4<sup>o</sup> Sur les enquêtes effectuées en 1905 dans les maisons signalées comme foyers de tuberculose (20 mars 1906).

(b) (1909, 7 pp.) Enquêtes effectuées en 1908 dans les maisons signalées comme foyers de tuberculose.

(c) (1909, 127 pp.) Recherches effectuées au Bureau du Casier Sanitaire pendant l'année 1908 relatives à la répartition de la tuberculose et du cancer dans les maisons de Paris.

- STANLEY, A. (1909). *Annual Report of the Shanghai Municipal Council (Health Department) for 1908.* Shanghai: Kelly and Walsh. 41 pp., 32 × 20 cm.

- SIMPSON, W. J. and others (II. 1909). *Report on Plague in the Gold Coast in 1908.* London: J. and A. Churchill, 7 Great Marlborough St. 55 pp. Price 2/- net. Boards. 33 × 25 cm.

*Third Report of the Wellcome Research Laboratories at the Gordon Memorial College, Khartoum.* Director, Andrew Balfour. Published for the Dept. of Education, Sudan Government, Khartoum, by Baillière, Tindall and Cox, 8 Henrietta St., Covent Garden, London, 1908. 477 pp., 48 plates, 218 text figures. 28 x 20 cm. Cloth. Price £1 1s. Od.

*Review of some of the recent advances in Tropical Medicine, Hygiene and Tropical Veterinary Science, with special reference to their possible bearing on Medical, Sanitary and Veterinary Work in the Anglo-Egyptian Sudan, being a supplement to the Third Report etc.* by Andrew Balfour and R. G. Archibald. 251 pp. (same size as the preceding), 1 map. Price 10/6.

This monumental report contains a large amount of valuable and original material. It includes papers by Leiper on parasitic worms, by Theobald on mosquitoes, by Werner on reptiles, by Waterston on Anthropology, by Balfour on trypanosomiasis, piroplasmosis and spirochaetosis, and on public health problems in Khartoum. Ensor and Archibald deal with Sleeping Sickness, Cummins and Bousfield with Kala-azar, King with Entomology, Wenyon with protozoology, etc. Numerous and for the most part excellent coloured plates and figures illustrate the volume. Whereas the First and Second Reports were distributed gratuitously a charge, which has been calculated as low as possible, will be made for the Third and subsequent Reports. The form in which the Report and Supplement are issued is excellent.

#### NEW PERIODICALS.

*The British Health Review*, vol. 1., No. 1 (15th April, 1909). Edited by Mrs. L. Hodgkinson. 36 pp., price 3d. monthly; 4/- per annum, post free. London: Published by British Health Review Co., 21 Paternoster Square, E.C.

It is difficult to understand to what class of readers the journal will appeal unless it be found among frequenters of vegetarian restaurants.

*Bulletin of the Pasteur Institute of Southern India.* No. 1, 1908. (Published under the authority of the Central Committee of the Association.) 34 pp. 33 x 21 cm. Madras, 1908: Printed by the Superintendent, Government Press.

Contains short notes by J. W. Cornwall and M. Kesava Pai on: The influence of cyllin on rabies virus—the diagnosis of rabies in inoculated animals—the measure of immunity in men and animals—the degree of infectivity of the various parts of the central nervous system—Negri bodies—rabies toxins—histology of the blood in rabies—susceptibility of guinea-pigs and their immunization—action of chloroform on fixed virus—the infectivity of peripheral nerves in rabies, etc.

*Hygiene and Physical Education*, April, 1909. Vol. 1., No. 2, 127 pp. Published by The F. A. Bassette Company, Springfield, Massachusetts. The second number, the first to reach us for review, contains:

Ten Commandments of Schoolhouse Construction by Wm. E. CHANCELLOR, Supt. of Schools, City of South Norwalk, Conn. (illustrated).—The Problems of Hygiene and the Province of the State Normal School by HOMER H. SEERLEY, LL.D., Iowa State Normal School.—School Hygiene and Efficiency by ARTHUR E. BENNETT, PH.D., Upper Iowa University.—School Hygiene and Community Health by JESSIE BENTON MONTGOMERY, Indiana State Normal

School.—The School and the Germ-Carrier by PROF. EDWIN O. JORDAN, University of Chicago.—Prophylaxis in the Practice of the School Superintendent by EDWIN L. STEVENS, L.H.D., City of New York.—A Lesson from Medical Inspection of Schools by GEO. H. MARTIN, LL.D., Secretary Massachusetts Board of Education.—American School Hygiene Association by Dr THOS. A. STOREY, Sec'y.—Function and Administration of Medical Supervision in the School by Dr J. E. RAYCROFT, University of Chicago.

*Sanidad y Beneficencia* Boletin Oficial de la Secretaria, Vol. I., No. 1 (appeared April 1909), 156 pp., 11 figs., 19 photographs. No. 2, pp. 157-339. Published by Dr E. B. Barnet under the direction of the officials Drs Matias Duque, Juan Guiteras and Juan M. Plá. Editorial Office in the Secretaria de Sanidad y Beneficencia, Habana, Cuba.

The opening number of this new monthly periodical contains: Notes in regard to yellow fever and tuberculosis in Cuba during the past years by J. GUITERAS.—The division of charities by J. M. PLA.—Tributo de gratitud (with portraits of all those concerned in the great discoveries concerning yellow fever) by E. B. BARNET.—Etiology of yellow fever and destruction of mosquitoes by A. AGRAMONTE.—Clinical Observations on the different varieties of rings obtained by the Heller reactive by E. F. RODRIGUEZ.—Statistics relating to Public Health and Demography in Cuba, Meteorological Reports, etc. The scientific papers appearing in the second number are: Symptomatology and diagnosis of yellow fever by J. GUITERAS.—Special diagnosis of yellow fever with reference to mild cases by M. S. LEBREDO.—Preliminary note on the agent of Trachoma by L. E. FINLAY and J. CARTAYA. The Journal is published in Spanish-English-French and presents an excellent appearance.

#### REPRINTS.

- BRAZIL, V. (1909). Serunitherapia anti-ophidica. *Revista Medica de S. Paulo*, No. 15, 13 figs., 43 pp. reprint.
- CLERMONT, G. (1909). Experimental research on the hypodermic and intra-venous injection of Vichy-water taken at the spring. *Centre Médical* (Riom). 14 pp. reprint.
- CRENDIPOULO, M. (1909). Une Fièvre des Pays chauds non encore décrite. *Presse Médicale*, No. 13, 16 pp.
- IAROTZKY, A. (1907). Lokale Erscheinungen bei passiver Immunität gegen Schweinerotlauf und Infektion. *Centralbl. f. Bakteriol.*, 1 Abt. Bd. XLIV., Orig. pp. 76-89, 7 figs.
- PINZANI, G. (1909). Proposta di un nuovo metodo per la determinazione della luce. *Rivista d' Ingegneria Sanitaria* (Biella), 7 pp. reprint.
- SEWASTIANOFF, DR (1909). Die phagozytäre Tätigkeit der roten Blutkörperchen und der Blutplättchen (Bizzozero). *Wien. klin. Rundschau*, Nos. 26-29, 19 figs., 14 pp.

## INDEX OF AUTHORS.

	PAGE
ARKWRIGHT, J. A. Varieties of the Meningococcus with special reference to a Comparison of Strains from Epidemic and Sporadic Sources . . . . .	104
ARKWRIGHT, J. A. On the Production of Antitoxin by the Injection of Filtrates of Cultures of Non-virulent Diphtheria Bacilli . . . . .	409
ARMIT, H. W. The Physiological Effect of Cobalt Carbonyl Vapour . . . . .	249
BLUE, R. Anti-plague Measures in San Francisco, California, U.S.A. . . . .	1
BOYCOTT, A. E. and HALDANE, J. S. The Progress of Ankylostomiasis in Cornwall . . . . .	264
BULLOCH, W. and CRAW, J. A. On the Transmission of Air and Micro-organisms through Berkefeld Filters. (Plate I. and One Text Figure) . . . . .	35
CRAW, J. A. On the Danyz Effect with reference to the Toxin-antitoxin Reaction. (One Figure) . . . . .	46
CRAW, J. A. <i>See</i> BULLOCH.	
DURHAM, H. E. Studies on Cider and Perry. No. I. Sulphite Preservatives . . . . .	17
GIMLETTE, J. D. Plague in Further India . . . . .	60
GOADBY, K. A Note on Experimental Lead Poisoning . . . . .	122
GLENNY, A. T. <i>See</i> SÜDMERSEN.	
HALDANE, J. S. <i>See</i> BOYCOTT.	
HALLIBURTON, W. D. The Bleaching of Flour . . . . .	170
HEWLETT, R. TANNER, VILLAR, SIDNEY, and REVIS, CECIL. On the Nature of the Cellular Elements present in Milk . . . . .	271
JOHNSTONE, JAMES. Routine Methods of Shellfish Examination with Reference to Sewage Pollution . . . . .	412
KAUFMANN, W. P. On the Absence of Respiratory Disorders in those inhaling Starch Dust over Long Periods . . . . .	220
LANE-CLAYPON, JANET E. Observations on the Influence of Heating upon the Nutrient Value of Milk as an Exclusive Diet for Young Animals. 1 Figure . . . . .	233
LANE-CLAYPON, JANET E. Multiplication of Bacteria and the Influence of Temperature and some other Conditions thereon. 1 Figure . . . . .	239

	PAGE
MACCONKEY, A. Further Observations on the Differentiation of Lactose-fermenting Bacilli, with special reference to those of Intestinal Origin . . . . .	86
MASSEY, R. E. A Comparison between the Germicidal Power of a Disinfectant in Solution and in the Emulsified State. (1 Text Figure) . . . . .	341
NASH, J. T. C. House Flies as Carriers of Disease . . . . .	141
NOON, L. Observations on the Evolution of Immunity in Disease. 15 Figures . . . . .	181
PAGE, CECIL H. W. British Industrial Anthrax. Part I. (1 Figure) . . . . .	279
PAGE, CECIL H. W. British Industrial Anthrax. Part II . . . . .	357
REVIS, CECIL. <i>See</i> HEWLETT.	
SCHRYVER, S. B. Some Investigations on the Toxicology of Tin, with Special Reference to the Metallic Contamination of Canned Foods . . . . .	253
SÜDMERSEN, H. J. and GLENNY, A. T. Variation in Susceptibility of Guinea-pigs to Diphtheria Toxin. (Seven Charts) . . . . .	399
SYMMERS, W. ST C. and WILSON, W. J. Some points bearing on the Bacteriology of Cerebro-spinal Meningitis . . . . .	9
TODD, C. An Anti-serum for Scorpion Venom . . . . .	69
VILLAR, SIDNEY. <i>See</i> HEWLETT.	
WILSON, A. "On the Relative Efficacy of the Doulton, Berkefeld and Brownlow Filters" . . . . .	33
WILSON, W. JAMES. On Heterologous Agglutinins more particularly those present in the Blood Serum of Cerebro-Spinal Fever and Typhus Fever Cases . . . . .	316
WILSON, W. J. <i>See</i> SYMMERS.	

## INDEX OF SUBJECTS.

	PAGE
Agglutination of <i>B. aquatilis alcaligenes</i> , <i>see</i> Cerebro-spinal	
"    " <i>B. coli</i> , <i>see</i> Cerebro-spinal, Typhus	
"    " <i>B. typhosus</i> , <i>see</i> Cerebro-spinal, Typhus	
"    " Meningococcus ... ... ... ... ... ...     108	
"    " tests in cerebrospinal fever ... ... ... ...     15	
"    " <i>see</i> pp. 185, 202	
Agglutinins, heterologous, in serum in cerebro-spinal meningitis, etc. ...     316	
Air, <i>see</i> Starch dust, Industrial	
Ankylostomiasis in Cornwall ... ... ... ... ...     264	
Anthrax among animals ... ... ... ... ...     307	
" bacteriology of ... ... ... ... ...     357	
" bristles and horsehair in relation to ... ... ...     296, 300	
" dangerous processes and materials ... ... ... ...     301	
" disinfection ... ... ... ... ...     362	
" industrial (British) ... ... ... ... ...     279, 357	
" serum therapy in ... ... ... ... ...     376	
" statistics ... ... ... ... ...     281	
" treatment ... ... ... ... ...     376	
Antitoxin for scorpion-venom ... ... ... ... ...     69	
"    "    " effects of ... ... ... ... ...     82	
"    "    " preparation and testing of ... ... ...     80	
" <i>see</i> Diphtheria	
Autoinoculation ... ... ... ... ...     213	
<i>Bacillus anthracis</i> , <i>see</i> Anthrax	
" <i>aquatilis alcaligenes</i> (=Bac. Grosvenor) ... ... ... ...     317	
" <i>see</i> Agglutination	
" <i>coli</i> , conditions influencing growth of ... ... ... ...     239 et seq.	
"    " isolation of, from shellfish ... ... ... ...     426	
"    " <i>see</i> Agglutination, Disinfection	
" <i>diphtheriae</i> , <i>see</i> Diphtheria	
" <i>enteritidis</i> , conditions influencing growth of ... ... ...     239 et seq.	
" <i>faecalis alkaligenes</i> , <i>see</i> Agglutination	

	PAGE
<i>Bacillus "Grosvenor," see Agglutination, Bacillus aquat. alc.</i>	
<i>" mycoides, see Disinfection</i>	
<i>" pestis, see Plague</i>	
<i>" pseudotuberculosis rodentium</i> ... ... ... ... ... ... 183	
<i>" " " agglutination experiments with</i> ... ... 202	
<i>" " " inoculation experiments with</i> ... ... 193	
<i>" " " sugar reactions of</i> ... ... 184	
<i>" typhosus, conditions influencing growth of</i> ... ... 239 et seq.	
<i>" " see Agglutination, Disinfection</i>	
<i>" see Lactose</i>	
<i>Bacteria, multiplication of, conditions influencing</i> ... ... ... 239	
<i>" see Sewage</i>	
<i>Bacteriology of meningitis</i> ... ... ... ... ... ... 9	
<i>" see Lactose, Indole, Fermentation-tests</i>	
<i>Bleaching of flour</i> ... ... ... ... ... ... 170	
<i>Bread, see Bleaching</i>	
<i>California, plague in</i> ... ... ... ... ... ... 1	
<i>Canned foods, metallic contamination of</i> ... ... ... ... ... 253	
<i>" " see Tin</i>	
<i>Cerebro-spinal meningitis</i> ... ... ... ... ... ... 9, 104	
<i>" " agglutination tests in</i> ... ... ... ... 316, 325	
<i>Cider and Perry, sulphite preservatives</i> ... ... ... ... ... 17	
<i>Cobalt carbonyl vapour, physiological effects of</i> ... ... ... ... 249	
<i>Complement, see Meningococcus</i>	
<i>Dairy, see Milk</i>	
<i>Danysz effect</i> ... ... ... ... ... ... 46	
<i>Diarrhoea, see Summer</i>	
<i>Diphtheria antitoxin, production by means of avirulent culture-filtrates</i> ... 409	
<i>" toxin, variation in susceptibility of guinea-pigs to</i> ... ... 399	
<i>Disease spread by flies, see Flies</i>	
<i>" see Immunity</i>	
<i>Disinfectants, effects in solution and in emulsion compared</i> ... ... ... 341	
<i>Disinfection experiments with phenol</i> ... ... ... ... ... 342	
<i>" in relation to Anthrax</i> ... ... ... ... ... 362	
<i>Dust, different kinds, in relation to respiratory disorders</i> ... ... ... 220	
<i>Evolution of immunity in disease</i> ... ... ... ... ... 181	
<i>Examinations for the U.S. Army Medical Corps</i> ... ... ... ... ... 356	
<i>Factories, see Dust</i>	
<i>Fermentation tests applied to intestinal bacilli</i> ... ... ... ... ... 91	
<i>" " " meningococci</i> ... ... ... ... ... 105	
<i>Filters (Berkefeld), passage of bacteria through</i> ... ... ... ... ... 35	
<i>" relative efficacy of different kinds</i> ... ... ... ... ... 33	
<i>Fleas in relation to plague</i> ... ... ... ... ... ... 5, 61	

	PAGE
Flies as carriers of disease ... ... ... ... ... ... ...	141
" " " literature on ... ... ... ... ...	160
" " carriers of summer diarrhoea ... ... ... ...	151
" measures against ... ... ... ... ...	162
Flour, bleaching of ... ... ... ... ...	170
Foods, <i>see</i> Milk, Flour, Flies (contaminating), Preservatives, Canned	
Germicides, <i>see</i> Disinfectants	
<i>Homolomyia canicularis</i> , <i>see</i> Flies	
Hymenolepis diminuta and nana, in rats ... ... ... ...	7
Immunity, evolution of in disease ... ... ... ...	181
" serum therapy in Anthrax ... ... ...	376
" <i>see</i> Agglutination, Antitoxin, Complement, Danysz effect, Diphtheria, Opsonins	
India, <i>see</i> Plague	
Indole reaction ... ... ... ...	91
Industrial Hygiene, <i>see</i> Anthrax, Lead, Starch, Dust, Cobalt, Ankylostomiasis, Tin	
Insects, <i>see</i> Flea, Fly	
International Congress on Hygiene and Demography, 1910 ... ...	347
" " British National Committee ... ...	353
" " Committee of Organization ... ...	348
" " Division into sections ... ...	352
" " Permanent International Commission ... ...	349
" " Regulations ... ...	350
Intestinal bacilli, <i>see</i> Lactose	
Lactose-fermenting bacilli, differentiation of ... ... ...	86
Lead poisoning, experimental ... ... ...	122
Meningitis, cerebro-spinal ... ... ... ...	9, 104
Meningococcus ... ... ... ...	9
" agglutination of ... ... ...	13, 108
" complement-fixation test ... ...	113
" different strains compared ... ...	104
" organisms liable to be confused with ... ...	117
Metals, <i>see</i> Toxicology	
<i>Micrococcus catarrhalis</i> ... ... ...	11
Milk, effect of heating on its nutrient value ... ...	233
" nature of cellular elements in ... ...	271
Mines, <i>see</i> Ankylostomiasis	
<i>Musca domestica</i> , breeding places of ... ...	142
" " development of ... ...	144
" " range of flight ... ...	145

	PAGE
<i>Musca domestica</i> , rearing experimentally	... ... ... ... ... 146
" " see Flies	
Opsonic index in cerebro-spinal meningitis	... ... ... ... ... 14
Opsonins, estimations of	... ... ... ... ... 185, 187
" importance of	... ... ... ... ... 192
Perry, see Cider	
Phagocytosis, see Opsonins	
Phenol, disinfection experiments with	... ... ... ... ... 342
Physiological effects of cobalt carbonyl vapour	... ... ... ... ... 249
tin salts	... ... ... ... ... 259
Plague in animals, see Rats, Squirrels	
" Further India	... ... ... ... ... 60
" San Francisco	... ... ... ... ... 1
" prevention	... ... ... ... ... 1, 66
Poisoning, see Lead	
Preservatives, see Cider and Perry	
Publications received	... ... ... ... ... 134, 252, 441
Rats and plague	... ... ... ... ... 4, 66
" parasites in	... ... ... ... ... 7
San Francisco, plague in	... ... ... ... ... 1
Scorpion-venom, action of on blood	... ... ... ... ... 77
" " mucous membranes	... ... ... ... ... 78
" " susceptible animals	... ... ... ... ... 75
" animals immune to	... ... ... ... ... 74
" anti-serum for	... ... ... ... ... 69
" preparation of	... ... ... ... ... 70
" characteristics of	... ... ... ... ... 72
" susceptibility of animals to	... ... ... ... ... 73
Sewage pollution of shellfish	... ... ... ... ... 412
Shellfish, sewage pollution of	... ... ... ... ... 412
Squirrels and plague	... ... ... ... ... 2
<i>Staphylococcus pyogenes aureus</i> , see Disinfection	
Starch dust, effects of inhaling	... ... ... ... ... 220
<i>Stomoxys calcitrans</i> , see Flies	
Summer Diarrhoea, relation to flies	... ... ... ... ... 151
Temperature, influence upon bacterial multiplication	... ... ... ... ... 239
Tin, contamination of foods by	... ... ... ... ... 257
" detection and estimation of in food and tissues	... ... ... ... ... 255
" in canned foods	... ... ... ... ... 253
" physiological experiments with	... ... ... ... ... 259
Toxicology, see Cobalt, Lead, Tin	
Toxin, see Diphtheria	
Toxin-antitoxin reaction	... ... ... ... ... 46

*Index of Subjects*

455

	PAGE
<i>Trichinella spiralis</i> in rats	7
<i>Trypanosoma lewisi</i> in rats	7
Typhus fever, agglutination tests in	316, 332
United States Army Medical Corps examinations	356
Venom, <i>see</i> Scorpion	
Worms, <i>see</i> Ankylostomiasis, <i>Hymenolepis</i> , <i>Trichinella</i>	



I. A. R. I. 75

**IMPERIAL AGRICULTURAL RESEARCH  
INSTITUTE LIBRARY  
NEW DELHI.**